Update on activities in ISO and CEN

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Relevant groups in ISO and CEN

ISO/TC34/SC9:
- ISO: International Standardisation Organisation
- TC34: Technical Committee 34 on Food products
- SC9: Subcommittee 9: Microbiology

CEN/TC275/WG6:
- CEN: European Committee for Standardisation
- TC275: Technical Committee 275 for Food analysis – Horizontal methods
- WG6: Working Group 6 for Microbiology of the food chain
Meetings

       - EURL-Salmonella workshop: 9 June 2016 (Saint Malo, France)

2017:  - EURL-Salmonella workshop: 29 & 30 May 2017 (Zaandam, the Netherlands)
       - annual meetings of ISO-SC9 and CEN-WG6: 19-23 June 2017 (Tokyo, Japan)
EN ISO 6579-1 Detection of *Salmonella*

- 1\textsuperscript{st} FDIS voting, 12 Nov 2015–12 Jan 2016: CEN: 100\% (20/20) pos. ISO: 96\% pos (24 pos; 1 neg) & ed. and technical comments
- 9 March – 20 April 2016: written consultation at ISO/TC34/SC9 and CEN/TC275/WG6 for agreement on introduction technical changes
- 20 June 2016: decision CEN that 2\textsuperscript{nd} FDIS voting is needed
- 2\textsuperscript{nd} FDIS voting 31/10/2016 – 26/12/2016: 31x positive, 1x negative, 11x abstention
- Small amount of (editorial) comments – introduced in final doc
- January – February 2017: review of 3 proofs
- 28 February 2017: **publication EN ISO 6579-1!**
- Information on development of EN ISO 6579-1 summarised in: Mooijman, K.A. The new ISO 6579-1: A real horizontal standard for detection of *Salmonella*, at last! Food Microbiology (2017), in press, available on line since 18-03-2017: [http://dx.doi.org/10.1016/j.fm.2017.03.001](http://dx.doi.org/10.1016/j.fm.2017.03.001)
Changes in EN ISO 6579-1(I)

General

- **Incorporation of ISO 6785** (milk and milk products) → after publication of EN ISO 6579-1, ISO 6785 has been withdrawn
- **Incorporation of Annex D of ISO 6579** (2007), samples for primary production stage
- Description of detection of *S. Typhi* and *S. Paratyphi* in normative annex D:
  - Use of Selenite Cystine (SC) broth, additional to RVS and MKTTn, for selective enrichment
  - Use of Bismuth Sulphite (BS) Agar, additional to XLD, for selective plating out
- For preparation of initial suspensions **referred to ISO 6887** series (‘Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Parts 1 to 6’)

Update ISO and CEN, May 2017
Changes in EN ISO 6579-1(II)

Non-selective pre-enrichment

- In composition of BPW, type of peptone has become less prescriptive:
  - ISO 6579:2002 indicates ‘enzymatic digest of casein’
- Range incubation temperature of non-selective media (like BPW) has become broader:
  - ISO 6579:2002 indicates 37 °C ± 1 °C
  - EN ISO 6579-1 indicates: incubate between 34 °C and 38°C
- In informative notes the possibility to refrigerate pre,- and selective enriched cultures for a maximum of 72 h is indicated
Changes in EN ISO 6579-1(III)

Selective enrichment

● Selective enrichment media:
  – First selective enrichment: choose either RVS broth or MSRV agar
  – Second selective enrichment: MKTTn
  – Primary production samples: only MSRV (like in Amd1 of ISO 6579, 2007)

● pH MKTTn amended:
  – ISO 6579:2002 (Cor.1:2004) indicates: pH base medium: 8,0 ± 0,2; complete medium to be used on day of preparation
  – EN ISO 6579-1 indicates: pH base medium: 8,0 ± 0,2. Complete medium: can be stored in the dark at 5 °C, but do not use if pH is <7
    › Based on info from 5 studies: good growth Salmonella at pH=7-8

● Incubation time of selective enrichment media retained for 24 h, except for:
  – Dried milk and cheese (2x 24 h)
  – Samples from primary production stage (on MSRV; 2x 24h if necessary)
Changes in EN ISO 6579-1(IV)

Plating-out and confirmation

- **XLD agar** is retained as mandatory first isolation medium
- Plating stage has been made less prescriptive: objective is to obtain well-isolated colonies. Informative note is added, suggesting the use of one large size or two normal size plates
- Tables are added in an annex to give clearer direction for the choice of suitable second plating media
- Confirmation on only one suspect colony (in stead of one colony of each medium combination). If negative, 4 more suspect colonies from different media combinations has to be tested
- Allowed to perform parallel biochemical testing and purity check
- The non-selective medium for purification has been left for choice

Update ISO and CEN, May 2017
Changes in EN ISO 6579-1(V)

Confirmation

- Two confirmation tests have become **optional**: β-Galactosidase test and indole reaction
- One confirmation test has been **deleted**: Voges-Proskauer reaction
- Details on serotyping moved to EN ISO 6579 part 3. In part 1 serological confirmation (to serogroup level) is described
- Table 1 (interpretation of biochemical tests) has been improved
Changes in EN ISO 6579-1(VI)

Performance characteristics

● Performance testing for quality assurance of media is added (currently in ISO 11133)
● New clause 11 on Accuracy (precision) of the method is added
● Performance characteristics amended and added (Annex C):
  – Accordance & concordance are removed
  – LOD$_{50}$ (Level of detection where probability of detection is 50%) added for food samples (calculated from raw data of validation study of 2000)
  – Performance characteristics added for analysing food samples on MSRV
  – Performance characteristics added for analysing samples from primary production stage on MSRV agar (based on 3 EURL-Salmonella studies: 2008, 2012, 2013)

Update ISO and CEN, May 2017
### Example performance characteristics EN ISO 6579-1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Blank</th>
<th>STM5&lt;sup&gt;a&lt;/sup&gt;</th>
<th>STM44&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SE7&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SE91&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participating collaborators</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Number of samples per collaborator</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Number of collaborators retained after evaluation of the data</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Number of samples retained after evaluation of the data</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Test portion size, in g</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Specificity, in %</td>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sensitivity per serovar and level, in %</td>
<td>—</td>
<td>96,8</td>
<td>100</td>
<td>67,4</td>
<td>100</td>
</tr>
<tr>
<td>LOD&lt;sub&gt;50&lt;/sub&gt; per serovar (95 % confidence interval), in cfu/test portion</td>
<td>—</td>
<td>1,0 (0,7 to 1,4)</td>
<td>4,3 (3,3 to 5,6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOD&lt;sub&gt;50&lt;/sub&gt; overall (95 % confidence interval), in cfu/test portion</td>
<td>—</td>
<td>—</td>
<td>2,5 (2,1 to 3,0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Chicken faeces samples were artificially contaminated with reference materials with the following strains and levels:

- *Salmonella Typhimurium* (STM) at a level of 5 cfu/test portion and a level of 44 cfu/test portion;
- *Salmonella Enteritidis* (SE) at a level of 7 cfu/test portion and a level of 91 cfu/test portion.
Introduction of EN ISO 6579-1 in labs

- Analysis of milk and milk products harmonised with other food products (selective enrichment was RVS and SC) – ISO 6785:2001 is withdrawn
- Introduction of ISO 6579-1: ‘Main changes in the document, compared to ISO 6579:2002 are considered as minor’ → little to no effect on the performance characteristics
- Each MS (may) need to discuss with own accreditation board if re-verification of performance characteristics in own lab is needed
- Discussion with Dutch accreditation board: re-verification for most labs not needed, only for specific cases, e.g.: in case a lab wants to use MSRV in stead of RVS but has no experiences with MSRV; in case up to now only ISO 6785 was followed for dairy products.
Draft ISO/TS 6579-4 PCR monoSTM

2016:
● Several draft version of ISO/TS 6579-4 prepared by Burkhard Malorny (Germany) and discussed at CEN-TAG3 November 2016
● May: presentation (& discussion) of activities at annual meetings ISO/TC34/SC9 and CEN/TC275/WG6
● November: Call for strains for validation study by EURL-Salmonella

2017:
● March: approximately 400 strains received by EURL. Thanks a lot! All strains also typed by EURL; in case of discrepancies between typing results NRL and EURL, typing will be repeated by EURL (approx. 25/400)
● May: meeting CEN-TAG3, discussion of next draft ISO/TS 6579-4
● June: presentation activities at annual meetings ISO and CEN

Update ISO and CEN, May 2017
Draft ISO/TS 6579-4 PCR monoSTM – next steps

- Selection of strains for verification of draft PCR protocols by EURL-Salmonella and NRL-Salmonella, Germany
- Fall 2017 (?): verification studies by EURL and NRL-Germany
- As soon as technical work is finished, movement from CEN-TAG3 to ISO-WG10
- Call for PCR experts for ISO-WG10 (by secretariat ISO/SC9)
- Launch New Work Item Proposal (NWIP) in ISO for a new part 4 of ISO 6579 (ISO/TS 6579-4) with tentative title: ‘Polymerase chain reaction (PCR) for identification of monophasic Salmonella Typhimurium (1,4,[5],12:i:-)’
- When final draft of ISO/TS 6579-4 is available → Organisation of an interlaboratory study for determining performance characteristics (2018/2019?)
Harmonisation of incubation temperature (I)

- In 2014 at meetings ISO/TC34/SC9 and CEN/TC275/WG6 agreed to use broader temperature range for incubation of non-selective media: 34-38 °C instead of 37 °C ± 1 °C (harmonisation with US).
- At lab of Adria, France (Daniele Sohier) experiments performed to test influence of incubation temperature on selective growth of Salmonella and several Enterobacteriaceae species. Conclusions:
  - no impact of incubation at 35 °C or at 37 °C on growth of Salmonella spp.
  - some impact on the growth of some (other) Enterobacteriaceae species
- In 2015 proposed to set up a protocol to test with members of ISO/CEN the possible broader temperature range for incubation of selective media. Especially test the influence on the growth of Enterobacteriacea.
Harmonisation of incubation temperature (II)

- Protocol drafted in 2016: culturing of ‘routine samples’, preferably with high amount of background flora (e.g. raw products, pps samples) and if possible with *Salmonella*, in MKTTn at 37 °C ± 1 °C and at 35 °C ± 1 °C. Incubation of Plating-out from MKTTn-37 °C also at 37 °C and plating-out from MKTTn-35 °C also at 35 °C.
- May-July 2016: call for participants for performing experiments among members of ISO and CEN → 7 countries agreed to perform some experiments.
- September 2016: laboratories invited to perform experiments, following updated protocol.
- March 2017, study results received from 7 laboratories. Data will be analysed in June 2017 (by EURL-*Salmonella* and Adria).
CEN mandate M/381 (history)

- **Aim:** ‘to have **standardised** and **validated** methods that are referred to in legislation in order to support the EU food policy’
- **Project started in 2007**
- **Concerned international standardisation and validation of 15 microbiological methods, including validation of the method for detection of *Salmonella* in samples from the primary production stage (pps)**
- **Performance characteristics for detection of *Salmonella* in pps samples determined from EURL-*Salmonella* interlaboratory studies of 2008 (chicken faeces), 2012 (pig faeces) and 2013 (boot socks – combined EURL/CEN-mandate study)**
Finalisation CEN mandate M/381

- CEN mandate project ends in June 2017. By then all 15 EN/ISO standards including the performance characteristics have to be published.
- Raw data of all studies have to remain available for possible future recalculation → will probably be stored (per study) at DG-Sante and CEN.
- Agreed at meeting of CEN 2016 to publish all validation studies in a special issue of the International Journal of food Microbiology; manuscripts have to be finalised before 30 June 2017.
  → All participants in validation studies shall be mentioned in the Acknowledgements.
Revision of ISO 6887 series: Microbiology of the food chain – Preparation of test samples

FDIS voting Oct-Dec 2016; final publication Jan-April 2017:

- Part 1: General rules for the preparation of the initial suspension and decimal dilutions (including info on pooling of samples and verification protocol for pooling)
- Part 2: Specific rules for the preparation of meat and meat products
- Part 3: Specific rules for the preparation of fish and fishery products
- Part 4: Specific rules for the preparation of miscellaneous products (e.g. animal feed, eggs, cocoa products, acidic products)

March 2017 start revision of:

- Part 5: Specific rules for the preparation of milk and milk products
Other subjects of possible interest (I)

- Revision of EN ISO 7218 - General requirements and guidance for microbiological examinations
  - Update with information/reference to ‘new’ techniques
  - Reduce calculation section (refer to Excel calculators)
  - Reduce equipment section

- Revision of ISO/TS 22117 - Specific requirements and guidance for proficiency testing by interlaboratory comparison:
  - To make document full standard (instead of Technical Specification), as TS is not recognised in some countries
  - Take into account new information on statistical aspects for PT schemes
  - To include PT schemes for viruses, parasites, primary production, yeasts and moulds, molecular methods
Other subjects of possible interest (II)

- CEN-TAG9 Pre-enrichment step
  - Aim to come to an optimal pre-enrichment medium for detection of several (Gram neg) pathogenic bacteria, to resuscitate stressed or damaged cells
  - Started development of a protocol to determine performance characteristics for the pre-enrichment step (e.g. in BPW)

- ISO-WG25 Development of an international standard, in (likely) three parts on whole genome sequencing (project leader USA):
  1. Wet laboratory sequencing and analysis of sequence data
  2. Validation of data and methods
  3. Metadata and sequence repository
Other subjects of possible interest (III)

- Proposal at 2016 meeting of ISO-SC9 to start standardisation of a method for molecular (PCR) serotyping of *Salmonella*. Agreed that US will send a method proposal, including validation data to SC9-secretariat to launch an enquiry (among SC9 members) on the need to standardise such a method. May 2017: enquiry not yet launched.

- ISO 16140 series: see presentation Paul in 't Veld

- Next annual meeting of ISO/TC34/SC9 and CEN/TC275/WG6 on 19-23 June 2016 in Tokyo, Japan
Thank you
Questions?