National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Update on activities in ISO and CEN

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EURL *Salmonella*

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

Next annual meeting: 8-12 July 2019, Milan, Italy

Draft Amd.1 EN ISO 6579-1:2017 – I

- After publication of EN ISO 6579-1 a mistake was detected in the composition of Selenite cystine broth in Annex D.3: L-cystine solution should be 10 ml instead of 100 ml.
- 22/12/2017 – 26/02/2018: Consultation of ISO/TC34/SC9 and CEN/TC275/WG6 members to check for any other errors.

  → Outcome positive for publication of a corrigendum, but few more remarks received which needed discussion.
- June 2018: Remarks discussed at annual meeting ISO-SC9 and CEN-WG6. Agreed to draft an amendment.

Draft Amd.1 EN ISO 6579-1:2017 - II

- Nov-Dec 2018: First draft Amd.1 sent to members ISO-SC9 and CEN-WG6 (draft Resolution no 842):
  - Request for agreement to skip CD (Committee Draft) vote and go immediate to DIS (Draft International Standard) voting;
  - Invitation to nominate active experts;
  - Invitation to comment to the draft.
- Dec 2018: Resolution 842 taken with 22 approvals, no disapproval and 11 abstentions and some comments. Seven experts nominated from 7 different countries.
- Jan-Feb 2019: 2 additional comments received (after the deadline).

Draft Amd.1 EN ISO 6579-1:2017 - III

- Feb-April 2019:
  - Comments incorporated in second draft Amd.1;
  - Second draft Amd.1 sent to expert group for comments;
  - Comments expert group incorporated in draft DIS Amd.1 and sent to secretariat ISO-SC9.
- 10 May 2019: Document sent to SC9 members to inform about the changes to the document and to invite them for DIS voting from 08-07-2019 to 30-09-2019.

  → Consultation of NRLs-*Salmonella*

DIS Amd.1 EN ISO 6579-1:2017 – Content - I

- Suggested title: 'Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC'
- For incubation of selective media the temperature range has been extended from 37 °C ± 1 °C to 34 °C to 38 °C (like for incubation of non-selective culture media) – throughout the document.

  The following note is added to 6.3 (incubator 34-38 °C):

  – 'The range 34 °C to 38 °C for incubation of media includes the use of incubators set at 35 °C ± 1 °C, 36 °C ± 2 °C or 37 °C ± 1 °C.'

DIS Amd.1 EN ISO 6579-1:2017 – Content - II

A comment was made about a mistake in the final concentration of $MgCl_2$ in MSRV agar (Annex B.4). This is corrected in Amd.1:

- The final concentration $MgCl_2$ in MSRV agar should be 10.9 g/l. However, the composition given in ISO 6579-1:2017 results in a final concentration of 14.9 g/l $MgCl_2$

→ In Annex B.4 (MSRV agar from individual ingredients) the concentrations of the ingredients of solution A, the base medium and complete medium have been corrected.

DIS Amd.1 EN ISO 6579-1:2017 – Content - III

- The status of Annex D ('Detection of *Salmonella* Typhi and Paratyphi') has been changed from normative to informative.

  →To prevent further confusion whether Annex D should always be followed or not. Annex D should only be followed if *S.* Typhi and *S.* Paratyphi is specifically sought.
- Correction of the concentration of L-Cystine solution in Selenite Cystine broth from 100 ml to 10 ml in 1 L base medium

Draft ISO/TS 6579-4 PCR monoSTM - I

- 2016-2017: Working Drafts (WDs) of ISO/TS 6579-4 drafted by Burkhard Malorny (NRL-*Salmonella* Germany) including 3 PCR protocols.
- PCR protocols needed to be tested → call for test strains.
- March 2017: after call for strains, approx. 400 strains received!
- Until fall 2017: typing of all strains by EURL and repetition of typing in case of discrepancies.
- 2018: selection of 172 strains (target and non-target strains) and all tested with the 3 PCR protocols by NRL-*Salmonella* in Germany (BfR) and by EURL-*Salmonella*.
- For selection of strains, information of ISO/DIS 16140-6 was used (validation confirmation and typing methods).
- Jan-Feb 2019: comparison results of BfR and EURL.

Draft ISO/TS 6579-4 PCR monoSTM - II

3 PCR protocols:

1. Multiplex real-time PCR assay; Primers and probes published by Maurischat et al. 2015, Int. Food Microbiol. 193:8-14.
2. Agarose gel-based multiplex PCR assay; Primers published by Tennant et al. 2010 PLoS Negl. Trop. Dis. Mar 9;4(3):e621'; and EFSA 2010, EFSA Journal, 8(10), p. 1826.
3. Agarose gel-based single target PCR assay; Primers published by Maurischat et al. 2015, Int. Food Microbiol. 193:8-14.

Results 172 strains tested with 3 PCR assays

Typing results of owner/EURL (A)	No of strains	Real-time PCR Concordance with A (no of strains)		Gel-based multiplex PCR (Tennant) Concordance with A (no of strains)		Gel-based single target PCR Concordance with A (no of strains)	
		EURL	BfR	EURL	BfR	EURL	BfR
monophasic STM	37	36	37	36	37	36	37
biphasic STM	33	25	26	27	30	26	26
Inconsistent mSTM/STM	32	14	15	28	29	17	15
other serotypes	43	40	43	40	43	40	43
other Enterobacteriaceae	27	27	27	27	27	27	27

- Strains with different results between EURL and BfR with same PCR assay will be retested with the 3 PCRs (summer 2019);
- Differences between PCRs: mainly STM with 'Tennant PCR' and monoSTM with 2 other PCR assays → retested with slide agglutination

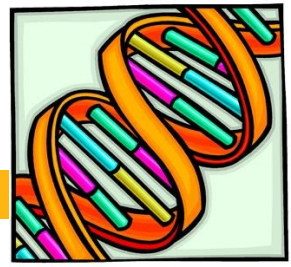
Draft ISO/TS 6579-4 PCR monoSTM next steps

- July 2019: Presentation results at annual meeting ISO-SC9 and CEN-WG6;
- Summer 2019: re-analysis of isolates with deviating results, with 3 PCR protocols and slide agglutination, by EURL-*Salmonella*.
- Fall 2019: Presentation of results of testing 172 strains, including the additional tests, in CEN-TAG3. Discussion of results and updating the PCR protocols, if necessary.
- End 2019/beginning 2020: launching New Work Item Proposal (NWIP) in ISO-WG10 and call for experts in WG10.

ISO 16140 Method validation parts 3-6

- Part 3: Protocol for the verification of reference and validated alternative methods implemented in a single laboratory
  - Additionally a Transition document is drafted for guidance on implementation of ISO 16140-3.
- Part 4: Protocol for single-laboratory (in-house) method validation
- Part 5: Protocol for factorial interlaboratory validation of non-proprietary methods
- Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures

- Pre-FDIS (Final Draft International Standard) voting: Sept-Nov 2018 for parts 4, 5, 6; March-May 2019 for part 3. All positive outcome.
- Results votings and comments will be discussed at meeting of ISO-WG3 in June and presented at annual meeting ISO-SC9 in July.
- Probable start FDIS voting all parts second half 2019.



Other subjects of possible interest

- April 2019: Publication of revised version EN ISO 22117 'Specific requirements and guidance for proficiency testing by interlaboratory comparison':
  - Document became full ISO instead of Technical Specification (TS);
  - Updates have been made to align the document with ISO 13528:2015 'Statistical methods for use in proficiency testing by interlaboratory comparison'

- ISO-WG25 WGS: 'Genomic sequencing of foodborne microorganisms – General requirements and guidance for bacterial genomes':
  - Voting New Work Item Proposal ISO 23418 spring 2018: approval to continue with document, but approx. 300 comments.
  - Voting of Committee Draft (CD) 16/05/2019 – 7/7/2019.
  - Draft Resolution to change title and scope 16/05/19 – 21/06/19

Any questions?