



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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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: 18-22 June 2018, Lausanne, Switzerland



EN ISO 6579-1:2017 Detection of *Salmonella*

After publication of EN ISO 6579-1 a mistake was detected in the composition of Selenite cystine medium (broth) in Annex D.3:

- Currently in D.3.1.3.1 the following is indicated:

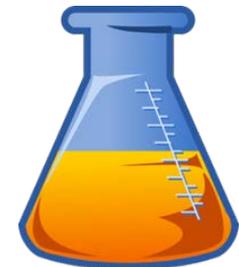
Base (D.3.1.1) 1000 ml

L-cystine solution (D.3.1.2) 100 ml

- This should be:

Base (D.3.1.1) 1000 ml

L-cystine solution (D.3.1.2) **10** ml



→ ISO/TC34/SC9 members have been consulted to check for any other errors (22/12/2017 – 26/02/2018). Outcome positive for publication of a corrigendum, but few more remarks received which need discussion.



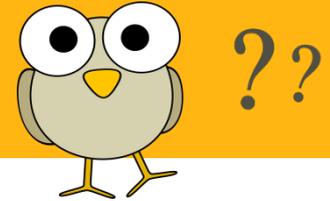
Questions on EN ISO 6579-1:2017 (I)

- Verification for introduction of ISO 6579-1 in the laboratory needed?
 - In principle no, but discuss with national accreditation body. Main changes, compared to ISO 6579:2002, are considered as minor' → little to no effect on the performance characteristics.
- Is it necessary to use two incubators, for 34-38 °C and 37 °C and is it necessary to report the exact temperature of 34-38 °C incubator?
 - No, for both. This range 34-38 °C was introduced to give more flexibility in the incubation temperature of non-selective media and to harmonise with USA. Any temperature between 34 °C and 38 °C is fine and it is not necessary to report this specifically. Easiest to use 37 °C incubator for all media.



Questions on EN ISO 6579-1:2017 (II)

- Is it demanded to use a 1 μ l loop for transfer of material from MSRV to XLD or can another size loop also be used?
 - Most important is to obtain single colonies. With a 1 μ l loop it is possible to obtain single colonies on a normal size (ca 9 cm) XLD plate. With a larger loop it may be the case that you need two normal size plates or one large plate (ca 14 cm) to obtain single colonies.
- Is it obligatory to apply Annex D (for detection of *S. Typhi* and *S. Paratyphi*) when testing routine samples?
 - No. The intention of this annex is to give (extra) guidance when *S. Typhi* or *S. Paratyphi* are specifically sought (e.g. in case of outbreaks). For the general analysis of samples from the food chain for the detection of *Salmonella* spp. (like 'normal' routine samples and PT samples) only the 'normal' procedure as described in the main document need to be followed.



Questions on EN ISO 6579-1:2017 (III)

- Is it obligatory to confirm *Salmonella* with poly H antiserum additional to confirmation with poly O antiserum?
 - Yes! The number of biochemical tests have been reduced and therefore polyvalent H-antisera was introduced, to be 'sure' that *Salmonella* is present. Some *Enterobacteriaceae* can give a positive reaction with polyvalent anti-O sera, but will give a negative reaction with polyvalent anti-H sera.
- How to interpret results in case of positive reaction with polyvalent anti-O sera but negative reaction with polyvalent anti-H sera (and typical biochemical reactions)?
 - According to ISO 6579-1:2017 this is presumptive *Salmonella*. Additional testing may be needed as it can also be another *Enterobacteriaceae*, or the polyvalent anti-H serum does not contain the H-factor(s) for the specific strain, or there may also be a small chance that the isolate concerns a (bi-)monophasic variant.



Draft ISO/TS 6579-4 PCR monoSTM

- Working Draft (WD) of ISO/TS 6579-4 drafted by Burkhard Malorny (NRL-*Salmonella* Germany) and contains 3 PCR protocols.
- WD need to be tested before further distribution → test strains needed.
- 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.
- Early 2018: further selection of 172 strains (target and non-target strains) and all are currently tested with the 3 PCR protocols by the NRL-*Salmonella* in Germany and by the EURL-*Salmonella*.
- Results 2 labs will be compared; WD ISO/TS 6579-4 updated if needed; further selection of strains for use in ILS for determining performance characteristics 3 PCR protocols.
- For selection of strains, information of ISO/DIS 16140-6 was used (validation confirmation and typing methods).



- Preferably non-human source;
- Of same MLVA types only 1-2 strains selected;
- Monophasic *Salmonella* Typhimurium (monoSTM) considered as the primary target strain and (biphasic) *Salmonella* Typhimurium (STM) considered as the additional target strain → following sets of (target) strains were selected:
 - Tested with serotyping and PCR as monoSTM (approx. 35 strains);
 - Tested with serotyping and PCR as STM (approx. 35 strains);
 - Tested with serotyping as monoSTM, but with PCR as STM (approx. 30 strains);
 - Tested with serotyping as STM, but with PCR as monoSTM (very rare, only 3 strains available);
- Non-target serovars: approx. 40 strains of different *Salmonella* serovars selected, including 'look-alikes' (e.g. *S. Agama* 4,12:i:1,6);
- Non-target genus strains: approx. 30 different strains from the family *Enterobacteriaceae* selected.

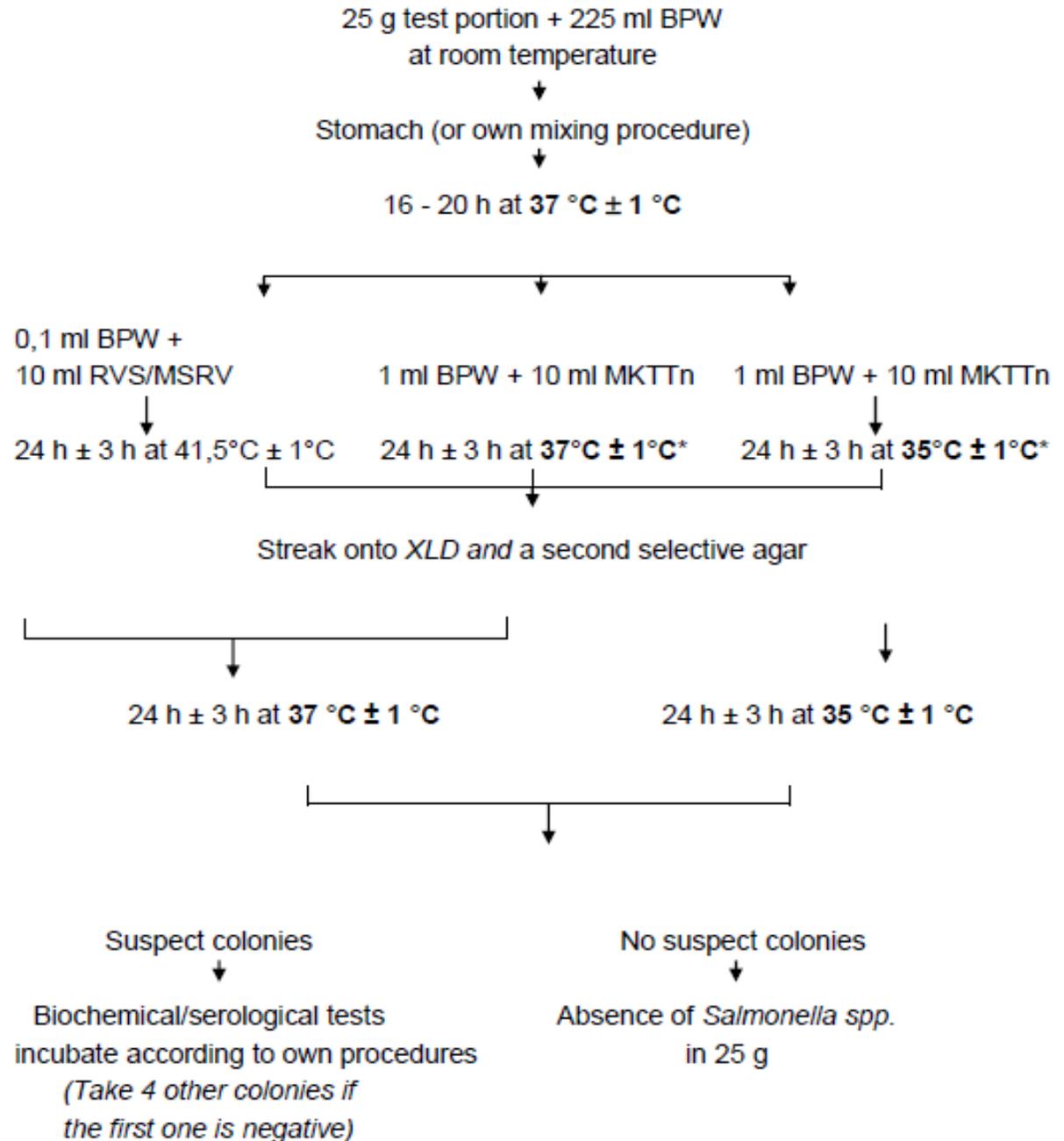


Harmonisation of incubation temperature

- In 2014 ISO/TC34/SC9 and CEN/TC275/WG6 agreed to use a broader temperature range for incubation of non-selective media: 34-38 °C instead of 37 °C \pm 1 °C (harmonisation with US).
- For incubation of selective media, temperature may be more critical → comparison studies 35 °C vs 37 °C needed.
- 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 \pm 1 °C and at 35 °C \pm 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.
- September 2016: members of ISO and CEN invited to perform experiments, following the protocol.
- June 2017: study results received from 9 laboratories, from 6 countries: Netherlands (3x), France, Egypt, Iran, USA, Thailand (2x); Total number of samples tested: 855 !

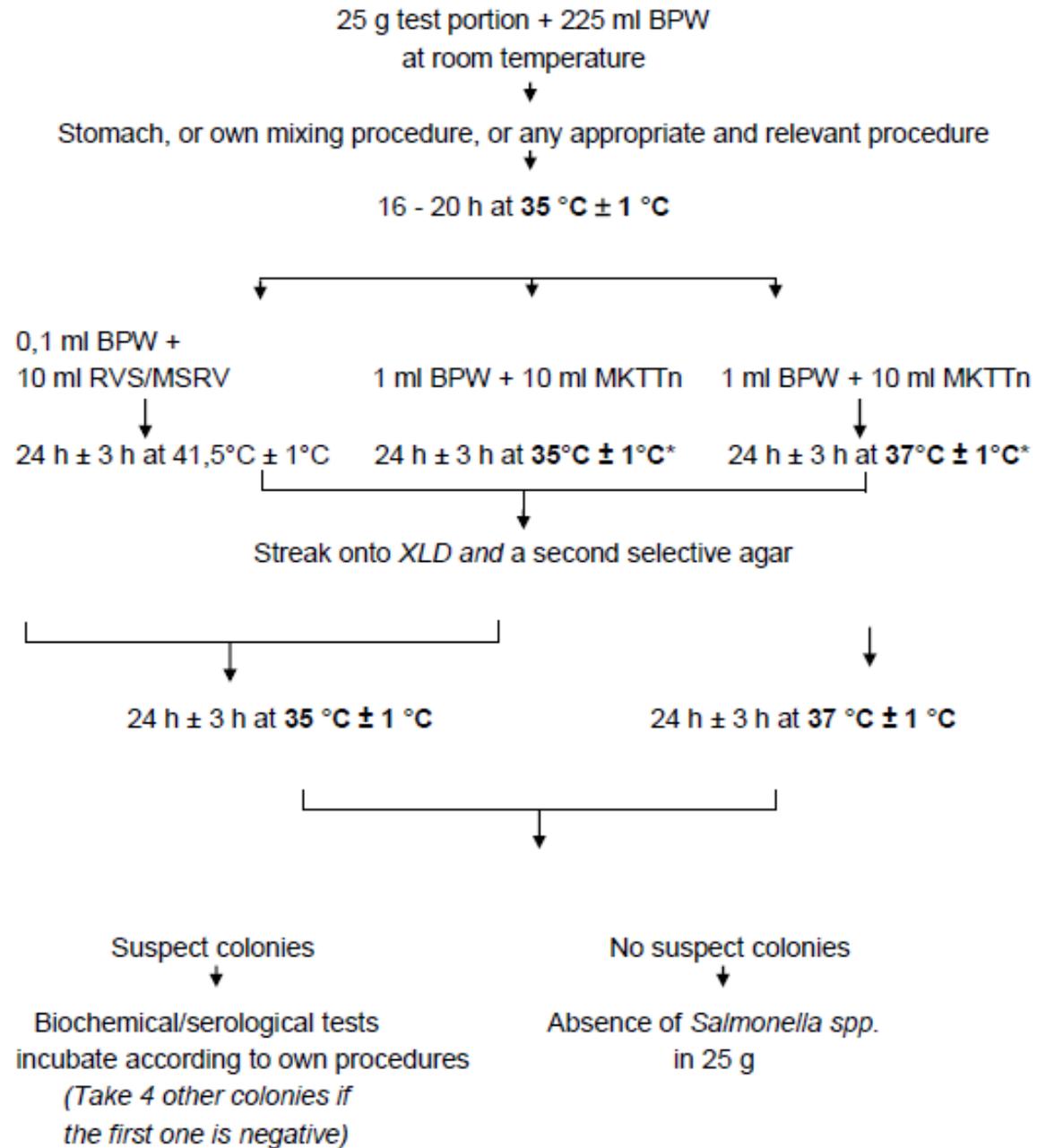
Study design
when BPW is
incubated at 37°C

Study was
designed to fit
with sensitivity
study of
ISO16140-2



Study design
when BPW is
incubated at 35°C

Study was
designed to fit
with sensitivity
study of
ISO16140-2





Data to be completed in extensive Excel sheet

Category	Reference method: ISO 6579											
	Characteristic colonies											
	Incubation at 41,5°C ± 1°C				Incubation at 37°C ± 1°C				Incubation at 35°C ± 1°C			
	RVS or MSRV / XLD (typical colonies)	RVS or MSRV / XLD (background flora)	RVS or MSRV / 2nd Agar Plate (typical colonies)	RVS or MSRV / 2nd Agar Plate (background flora)	MKTTn / XLD (typical colonies)	MKTTn / XLD (background flora)	MKTTn / 2nd Agar Plate (typical colonies)	MKTTn / 2nd Agar Plate (background flora)	MKTTn / XLD (typical colonies)	MKTTn / XLD (background flora)	MKTTn / 2nd Agar Plate (typical colonies)	MKTTn / 2nd Agar Plate (background flora)
	Salmonella plate 1	background	Salmonella plate 2	Background	Salmonella plate 3	Background	Salmonella plate 4	Background	Salmonella plate 5	Background	Salmonella plate 6	Background
Dairy	+++	+	+++	-	+++	++	+++	-	+++	++	+++	-
Dairy	+++	-	+++	-	+++	++	+++	-	+++	++	+++	-
Dairy	+ (2)	-	+ (4)	-	-	++	-	-	++	-	-	-
Dairy	+++	-	+++	-	+++	-	+++	-	+++	-	+++	-
Egg	+ (7)	-	+ (9)	-	+++	-	+++	-	+++	-	+++	-
Egg	+++	-	+++	-	+++	-	+++	-	+++	-	+++	-

→ Information aksed on:

- Pos/neg results for *Salmonella*, and confirmation results
- 'Amount of growth' of *Salmonella* at different temperatures (with 1-3 +)
- 'Amount of growth' of background flora at different temperatures



Results comparison studies

- In 3/9 laboratories samples analysed after pre-enrichment in BPW at 37 °C and after pre-enrichment in BPW at 35 °C
- Product categories tested:
 - Dairy products
 - Meat
 - Poultry products
 - egg products
 - vegetables/fruits
 - chocolate, bakery products
 - animal feed
 - samples from the primary production stage
 - waste water
 - Environmental samples food/feed production



Data interpretation

	37 C pos	37 C neg
35 C pos	PA	PD
35 C neg	ND	NA

PA: Positive Agreement
 NA: Negative Agreement
 ND: Negative Deviation
 PD: Positive Deviation

Sensitivity ref method MKTTn-37 C: SEref	$(PA + ND) / (PA + ND + PD) * 100\%$
Sensitivity alt method MKTTn-35 C: SEalt	$(PA + PD) / (PA + ND + PD) * 100\%$
Relative trueness, RT	$(PA + NA) / (PA + NA + ND + PD) * 100\%$

ISO 16140-2: 60 samples to be tested per category; ideally fractional positive results; each category at least 30 samples positive by reference and/or alternative method



Sensitivity (SE) and Relative Trueness (RT)

	SE-37 °C (%)	SE-35 °C (%)	RT (%)
BPW incubated at 37 °C	98,9	97,8	99,1
BPW incubated at 35 °C	92,3	100	98,1
BPW incubated at 37 °C <u>and</u> at 35 °C	97,5	98,3	98,8



Amount of background flora

	Selective media incubated at 37 °C ^a	Selective media incubated at 35 °C ^a
BPW incubated at 37 °C	1103	1071
BPW incubated at 35 °C	205	208
BPW incubated at 37 °C <u>and</u> at 35 °C	1308	1279

^a Total number of '+' reported by participants

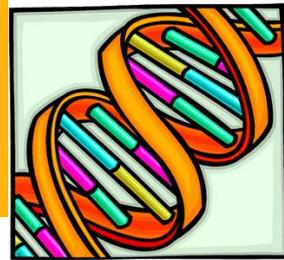


ISO 16140 Method validation parts 3-6

DIS voting 15/12/2017 – 09/03/2018 of

- Part 3: Protocol for the verification of reference and validated alternative methods implemented in a single laboratory
- 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

Results votings and comments discussed at meeting of ISO-WG3, Helsinki, Finland 23-25 May 2018.



Other subjects of possible interest

- Revision of EN ISO 7218 'General requirements and guidance for microbiological examinations': no information on progress.
- Revision of ISO/TS 22117 'Specific requirements and guidance for proficiency testing by interlaboratory comparison': DIS voting 07/02/2018 – 02/05/2018.
- CEN-TAG9 QC of pre-enrichment broth
 - Last meeting 17-18 April 2018;
 - Preparation of draft protocol to test pre-enrichment broth with stressed strains → request members ISO/CEN to test protocol.
- ISO/NP 23418 'Genomic sequencing of foodborne microorganisms – General requirements and guidance for bacterial genomes': Voting New Work Item Proposal 20/02/2018 – 09/05/2018.





Any questions?

