Salmonella spp Q-PCR test by AQMC

*M*ethod for rapid detection in raw milk

*S*almonellas are enterobaceria of *Salmonella* genus, pathogenic for man and presenting a strong contagiousness. They are responsible for gastroenteritis, food poisoning and typhoid or paratyphoid fevers (Colin M.P. and Lailler M.R., 2009; Korsak N. and al., 2004). Many methods, validated by AFNOR*, are commercialized on the market for the detection of *Salmonella* spp., however, it turns out that none of these methods are adapted to the specific case of raw milk cheese production, where the response time lag is a crucial problem. The method proposed today by the AQMC laboratory is an innovative *Salmonella* spp. detection method in raw milk, reliable and especially **rapid**: results in less than 11h. Moreover, the sample volume can be adapted to the user needs. This speedy diagnostic allows the release of raw milk before the rennet process and therefore the milk may be oriented depending on its hygienic quality.

*AFNOR : French Standardization and Certification Association (equivalent to the American ASTM)

Principle

Phase 1 ≈ 7h30 — **Enrichment in liquid media**

Possible stop point

Sample drawing ***(5 mL)***

Phase 2 ≈ 50 min — **Bacteria capture on magnetic beads + Apolipoprotein H**

Magnetization / Concentration

Bacteria pellet

Possible stop point

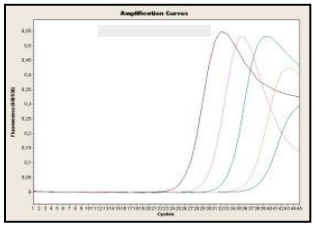
Phase 3 ≈ 40 min — **DNA preparation**

Bacteria lysis

Centrifugation

DNA

Phase 4 ≈ 1h40 — **Amplification and result analysis**



Rapid
Reliable
Robust (6 months at -20°C)
User-friendly
Ready to use

Results in less than 11h

Inclusivity

➢ Inclusivity was assessed on **56** *Salmonella* strains, including:

- 38 serotypes of the subspecies *Salmonella enterica enterica*
- 13 strains of other subspecies (*enterica arizonae, enterica diarizonae, enterica houtenae, enterica salamae, enterica indica, bongori)*
- 5 strains of non-motile variants *Salmonella enterica enterica typhimurium*

Number of tested strains	Results
56	+

➢ All strains were amplified with the AQMC Q-PCR *Salmonella* spp. method.

Number of tested strains	Results
21	–

Exclusivity

➢ Exclusivity was assessed on a appropriate range of **21** non-targeted pathogens: the method shows no cross-reactions.

Detectability

➢ Detectability was evaluated by comparing to the reference method ISO 6579:2002 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella spp*.

CFU/25mL sample	1000 cfu	100 cfu	10 cfu	1 cfu	Control
AQMC method	+	+	+	+	–
ISO 6579:2002	Presence	Presence	Presence	Presence	Absence

➢ Concordance of both methods for infection levels down to 1 CFU per 25 mL sample.

Conclusion

The AQMC Q-PCR *Salmonella* spp. method is presented in form of a ready-to-use diagnostic kit complying with precise criteria: *Salmonella* spp. presence/absence test ideally in less than 11 hours.

Kit composition

- **Diluant 1 (DL1): 25 mL vial**
- **ApoH beads: 1 mL tube**
- **Lysis buffer 1 (TL1): 20 mL vial**
- **Lysis buffer 2 (TL2): 2 mL vial**
- **Mix *Salmonella* 1 (M1 Salmonella): 1.4 mL tube**
- **Mix *Salmonella* 2 (M2 Salmonella): 200 µL tube**
- **PCR positive control: 75 µL tube**
- **PCR negative control: 75 µL tube**

Kit for 100 tests

AQMC
Analyses Qualité Microbiologie Conseil

Parc Agropolis II – 2196 Parc de la Lironde – Bât. 2
34397 Montpellier Cedex 5
Web : www.aqmc.fr
E-mail : laboratoire@aqmc.fr