TALLER TEÓRICO PRÁCTICO SOBRE VALIDACIÓN Y SU APLICABILIDAD EN MÉTODOS DE ANÁLISIS

GRUPO_____

Teniendo en cuenta lo visto en la presentación, escoja una de las dos metodologías anexas, revise la normatividad asociada al producto QUESO FRESCO y responda:

1- ¿Cuáles son las necesidades del CLIENTE?
2- ¿Cuál es el principio del método escogido?
3- Cuales reactivos y materiales de referencia se usaran en el método escogido.
4- Cuales equipos e instrumentos se usaran para este método.
5- ¿Teniendo en cuenta que la matriz a analizar es QUESO FRESCO el método escogido como se clasifica?, ¿Normalizado?, ¿no normalizado?
6- ¿De las guias vistas en la presentación cual escogería como guía para realizar la validación del método elegido? Justifique su respuesta.

reading, and the test value is the ratio of RFV and the standard. Results are interpreted after the test values and controls are compared to thresholds stored in the computer (Table **2001.09E**).

Before each new lot of reagents is used, specifications (or master factory calibration curve data) must be entered into the instrument using the master lot entry card. If this operation is not performed before initiating the tests, the protocol will not run.

Calibration, using the standard provided in the kit, must be performed upon receipt of a new lot of reagents after the master lot data has been entered. Recalibrate every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.

A positive result must be confirmed using enrichment and post-enrichment broths according to standard cultural procedures. Confirm typical or suspect colonies from each plate as in **967.26C** (*see* 17.9.02), **967.27** (*see* 17.9.03), and **967.28** (*see* 17.9.07). As an alternative to conventional tube system for *Salmonella,* any AOAC-approved commercial biochemical kits may be used for presumptive generic identification of foodborne *Salmonella* as described in **978.24** (*see* 17.9.04), **989.12** (*see* 17.9.05), and **991.13** (*see* 17.9.06).

References: *J. AOAC Int.* **85**, 609(2002); **87**, 390(2004)

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17.9.31

AOAC Official Method 2002.10 *Salmonella* Detection in Fresh Cheese, Dried Egg Products, and Fresh Chilled and Frozen Poultry

First Action 2002
Final Action 2006

ISO 6579:2002

(Applicable for the detection of *Salmonella* in fresh cheese, dried egg products, and fresh chilled and frozen poultry.)

See Table **2002.10** for results of the interlaboratory study supporting acceptance of the method.

Note: Selective enrichment combination Muller-Kauffmann tetrathionate broth/Rappaport-Vassiliadis soya broth may not be effective for the recovery of *S. typhi* and *S. paratyphi* from foods.

A. Principle

Salmonella are resuscitated under nonselective conditions and then propagated through the use of selective enrichment broths to levels that can be successfully recovered when isolated on selective agars.

B. Apparatus

(a) *Masticator.*—Stomacher (masticator), or equivalent, for homogenizing test portions.

(b) *Masticator bags, sterile.*—Appropriate capacity to accommodate test portions and masticator used.

(c) *Top-loading balance.*—Capacity of 2000 g with readability of 0.1 g.

(d) *Incubator.*—Maintaining 35–37°C.

(e) *Water baths.*—Maintaining 41.5 ± 1.0°C.

(f) *Sterile culture tubes with rack.*—16 × 150 mm tubes.

(g) *Syringe with filter.*—10 mL sterile plastic syringe with 0.2 µm filter.

(h) *Pipets.*—Sterile glass or plastic pipets, 1 mL with 0.01 mL graduations; 5 and 10 mL with 0.1 mL graduations.

(i) *Vortex mixer.*—For mixing tube contents.

(j) *Sterile inoculating loops.*—Ca 3 mm id or 10 µL, nichrome, platinum-iridium, or sterile plastic.

C. Media and Reagents

(a) *Buffered peptone water (BPW).*—Suspend 10 g peptone, 5.0 g NaCl, 9.0 g $Na_2HPO_4 \cdot 12H_2O$, and 1.5 g KH_2PO_4 in 1 L water, and mix thoroughly. Dispense 225 mL aliquots in 500 mL containers. Autoclave for 15 min at 121°C. Final pH should be 7.0 ± 0.2.

(b) *Rappaport-Vassiliadis soya peptone broth (RVS).*—Medium may be made from individual ingredients or from ISO-compliant commercial formulation. Prepare the following solutions: *Solution A.*—Dissolve 5.0 g soya peptone, 8.0 g NaCl, 1.4 g KH_2PO_4, and 0.2 g K_2HPO_4 in 1 L water. Heat to ca 70°C to completely dissolve medium. Prepare solution A on the day that complete medium is to be made. *Solution B.*—Dissolve 400 g $MgCl_2 \cdot 6H_2O$ in 1 L water. Solution B can be stored at room temperature in a dark bottle up to 1 year. *Solution C.*—Dissolve 0.4 g malachite green oxalate in 100 mL water. Solution C can be stored at room temperature in the dark for up to 6 months. To prepare complete RVS broth, combine 1000 mL Solution A, 100 mL Solution B, and 10 mL Solution C. Dispense complete medium in 10 mL aliquots into 16 × 150 mm tubes and autoclave 15 min at 115°C. Final pH should be 5.2 ± 0.2.

(c) *Muller-Kauffmann tetrathionate broth + novobiocin (MKTT+n).*—Suspend 4.23 g meat extract, 8.45 g tryptone, 2.54 g NaCl, 38.04 g $CaCO_3$, 30.27 g $Na_2S_2O_3$ (anhydrous), 4.75 g ox bile, and 9.5 mg brilliant green in 1 L water. Boil gently for 1 min. Cool below 45°C and store at 5–8°C. The base solution should be pH 7.0 ± 0.2. Prepare I–KI solution by dissolving 25 g KI in 25 mL water, adding 20 g resublimed I, dissolving, and diluting to 100 mL with sterile water. Prepare novobiocin solution by dissolving 0.04 g novobiocin sodium salt in 5 mL water. Filter-sterilize through 0.2 µm filter. On the day the medium is used, add 20 mL I–KI solution and 5.0 mL novobiocin per 1 L basal broth. Resuspend precipitate by gentle agitation, and aseptically dispense 10 mL portions into 16 × 150 mm sterile test tubes. Do not heat medium after addition of I–KI and novobiocin solutions.

(d) *Xylose lysine desoxycholate (XLD) agar.*—*See* **967.25A(d)** (*see* 17.9.01).

(e) *Second selective agar.*—The second agar used is at the discretion of the analyst. The agar used should be complementary to XLD and appropriate for isolation of lactose-positive strains of *Salmonella, S. typhi,* and *S. paratyphi.*

(f) *Nutrient agar.*—Suspend 3.0 g meat extract, 5.0 g peptone, and 15 g agar in 1 L water, and mix thoroughly. Heat to boiling to

Table 2002.10 Interlaboratory study results for detection of *Salmonella* in foods by ISO 6579 and AOAC 2000.06 and 995.20 culture methods

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dissolve completely. Autoclave at 121°C for 15 min. Cool in water bath, and pour 20 mL portions into 15 × 100 mm Petri dishes. Let agar cool and dry before use. Final pH should be 7.0 ± 0.2.

(g) *Triple sugar iron agar (TSI).*—Suspend 3.0 g meat extract, 3.0 g yeast extract, 20.0 g enzymatic digest of casein, 5.0 g NaCl, 10.0 g lactose, 10.0 g sucrose, 1.0 g glucose, 0.3 g iron(III) citrate, 0.3 g $Na_2S_2O_3$, 0.024 g phenol red, and 13 g agar in 1 L water, and mix thoroughly. Heat to boiling to dissolve completely. Dispense medium into 16 × 150 mm tubes, 1/3 full, and cap or plug to maintain aerobic conditions during use. Autoclave tubes for 15 min at 121°C. Before the medium solidifies, place tubes in a slanted position to form deep butts (ca 3 cm) and adequate slants (ca 5 cm) on solidification. The final pH should be 7.4 ± 0.2.

(h) *Urea agar.*—Suspend 1.0 g peptone, 1.0 g glucose, 5.0 g NaCl, 2.0 g KH_2PO_4, 0.012 g phenol red, and 15 g agar in 1 L water. Heat to boiling to dissolve completely. Autoclave for 15 min at 121°C. Cool to 50–55°C. Prepare urea solution by dissolving 400 g urea in water and dilute to a final volume of 1 L. Sterilize by filtration through 0.2 µm filter. To prepare complete medium, aseptically add 50 mL urea solution to 950 mL cooled urea agar base. Mix thoroughly. *Note*: Do not heat the complete medium. Dispense complete medium in 10 mL quantities into sterile tubes. Let tubes set in sloping position. The final pH should be 6.8 ± 0.2.

(i) *L-lysine decarboxylation medium (Falkow).—See* **967.25A(m)**(*2*) (*see* 17.9.01).

(j) *Bromocresol purple solution.*—0.2%. Dissolve 0.2 g in sterile water, and dilute to 100 mL.

(k) *Sterile physiological saline solution.—See* **940.36B(c)** (*see* 17.1.02).

(l) *Salmonella polyvalent somatic (O) antiserum.*—Antiserum A-I and Vi.

(m) *Salmonella polyvalent flagellar (H) antiserum.*—Poly A-Z.

D. Preparation of Test Suspensions

(a) *Fresh cheese.*—Aseptically weigh 25 g test portion into stomacher bag. Add 225 mL prewarmed (35°C) BPW, **C(a)**, and homogenize in stomacher for 1–3 min. Incubate the test suspension for 16–20 h at 35–37°C.

(b) *Dried egg products.*—Aseptically weigh 25 g test portion into stomacher bag. Add 225 mL BPW, **C(a)**, and homogenize in stomacher for 1–3 min. Incubate test suspension for 16–20 h at 35–37°C.

(c) *Poultry products.*—Aseptically weigh 25 g test portion into stomacher bag. Add 225 mL BPW, **C(a)**, and homogenize in stomacher for 1–3 min. Incubate test suspension for 16–20 h at 35–37°C.

E. Isolation

(a) *Growth in selective broth.*—Gently shake incubated test suspension, **D**, and transfer 0.1 mL into 10 mL RVS medium, **C(b)**, and an additional 1.0 mL into 10 mL MKTT+n broth, **C(c)**. Incubate RVS medium at 41.5 ± 1°C for 24 ± 3 h. Incubate MKTT+n broth at 35–37°C for 24 ± 3 h. Mix on a Vortex mixer all selective tubes. Streak loopful of incubated RVS medium onto selective enrichment plates of XLD agar, **C(d)**, and the second agar selected [*see* **C(e)** for details]. Repeat isolation with 3 mm loopful of MKTT+n test broth. Incubate plates 24 ± 3 h at 35–37°C. If growth is slight or if no typical colonies of *Salmonella* are present, reincubate at 35–37°C for additional 24 h. Reexamine plates for typical colonies of *Salmonella.*

(b) *Appearance of typical Salmonella colonies on XLD.*—Pink colonies with or without black centers. Many *Salmonella* may have large, glossy black centers or may appear as almost completely black colonies. Atypically, a few *Salmonella* cultures produce yellow colonies with or without black centers.

F. Treatment of Colonies

(a) *Inoculation of TSI.*—Pick with a sterile needle two or more typical or suspicious colonies, if present, from each XLD plate and second agar plate. Inoculate TSI slant, **C(g)**, by streaking agar slant and then stabbing the butt. Store picked selective plates at 5–8°C. Incubate slants at 35–37°C for 24 ± 2 h. Cap tubes loosely to maintain aerobic conditions while incubating slants to prevent excessive H_2S production. *Salmonella* cultures typically have alkaline (red) slant and acid (yellow) butt, with or without H_2S (blackening of agar) in TSI.

(b) *Inoculation of L-lysine decarboxylation medium.*—Using a sterile needle, transfer a portion of TSI culture to L-lysine decarboxylation medium, **C(i)**. Close tube caps tightly after inoculation and incubate at 35–37°C for 24 ± 2 h. *Salmonella* spp. give purple color of alkaline reaction throughout broth (final color is slightly darker than original purple color of medium). Sometimes tubes that are yellow after 8–12 h of incubation change to purple later. Negative test is permanently yellow throughout broth. If medium appears to be discolored (neither purple nor yellow), add a few drops of 0.2% bromocresol purple dye, **C(j)**, and reread the reaction.

(c) *Selection for identification.*—Retain all presumptive positive *Salmonella* cultures on TSI (alkaline slant and acid butt) agar for biochemical and serological test whether or not corresponding lysine decarboxylation reaction is positive (alkaline) or negative (acid). Do not exclude a TSI culture that appears to be non-*Salmonella* if the reaction in L-lysine decarboxylation broth is typical for *Salmonella.*

Treat these cultures as presumptive positive and submit them to further examination. Lysine decarboxylation medium is useful in detection of *S. arizonae* and atypical *Salmonella* strains that utilize lactose and/or sucrose. Discard only apparent non-*Salmonella* TSI cultures (acid slant and acid butt) if corresponding lysine decarboxylation broth is not typical (acid) for *Salmonella.* Test retained TSI cultures as directed in **F(d)** to determine if they are *Salmonella* spp., **967.27D(e)**(*1*) (*see* 17.9.03), or *S. arizonae* organisms, **967.27D(e)**(*2*) (*see* 17.9.03). If TSI slants fail to give typical *Salmonella* reactions, pick additional suspicious colonies from selective medium plate not giving any presumptive positive cultures, and inoculate TSI and lysine decarboxylation broth as in **F(a)** and **(b)**.

(d) *Identification.*—Apply biochemical and serological identification tests to three presumptive positive TSI cultures picked from selective agar plates streaked from RVS medium and to three presumptive positive TSI cultures picked from selective agar plates streaked from MKTT+n broth as directed in **967.27** (*see* 17.9.03) and **967.28** (*see* 17.9.07). Examine minimum of six TSI and six lysine decarboxylation broth cultures for each 25 g test portion tested. Any AOAC-approved *Salmonella* biochemical identification test kit may be used instead of performing the individual biochemical tests presented in this method.

Reference: *J. AOAC Int.* **86**, 275(2003)