

PREGLJED DOSADAŠNJIH I BUDUĆIH AKTIVNOSTI NRL-a CPS

Radionica RH NRL-a za

E. coli, Campylobacter, Salmonella, Listeria monocytogenes i Koagulaza pozitivne stafilokoke

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EURL NRL

European Union Reference Laboratory for Coagulase Positive Staphylococci (EURL)

– sukladno Uredbi (EU) 2017/625

French agency for food, environmental and
occupational health safety,

[ANSES](#) - [Laboratory for Food Safety](#)

Nacionalni referentni laboratorij (NRL)

Hrvatski veterinarski institut, Laboratorij za mikrobiologiju hrane

Hrvatski zavod za javno zdravstvo, Odsjek za mikrobiologiju namirnica i POU

The screenshot shows the website for the EURL Coagulase Positive Staphylococci. At the top, there are logos for the European Union, ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail), and the Hrvatski veterinarski institut (HVI) and HZJZ (Hrvatski zavod za javno zdravstvo). The main navigation menu includes: HOME, PRESENTATION, ACTIVITIES, WORKING AREAS, DOCUMENTS, USEFUL INFORMATION, and CONTACT. A featured section titled "WORKSHOP" highlights the "17TH WORKSHOP - GENERAL SCOPE" with a button for "ALL THE WORKSHOPS". Below this, the title "EURL COAGULASE POSITIVE STAPHYLOCOCCI" is displayed. A text block states: "On May 2006, the European Commission officially designated the French agency for food, environmental and occupational health safety, ANSES, as the European Union Reference Laboratory (EURL) for Coagulase Positive Staphylococci. The activities of the EURL are handled by ANSES's Laboratory for Food Safety."

Trenutni propisi i zahtjevi (EURL)



Uredba (EZ) br. 2073/2005 od 15. studenoga 2005. o mikrobiološkim kriterijima za hranu (s iz

- Kriteriji higijene procesa
- Kriteriji sigurnosti

CPS (koagulaza-pozitivni stafilocoki) – kriterij higijene procesa u mlijeku i mliječnim proizvodima, oljuštenim, kuhanim rakovima i školjkama

Stafilokokni enterotoksini – kriterij sigurnosti u mlijeku i mliječnim proizvodima.

Metode detekcije i brojanja

Brojanje CPS: HRN EN ISO 6888-1/-2/-3

- HRN EN ISO 6888-1/-2 Horizontalna metoda određivanja broja koagulaza-pozitivnih stafilocoka (*Staphylococcus aureus* i ostale vrste) - 1. dio: Postupak primjene Baird-Parker agara / - 2. dio: Postupak primjene agara s fibrinogenom plazme kunića
- HRN EN ISO 6888-3 – Horizontalna metoda za brojenje koagulaza-pozitivnih stafilocoka (*Staphylococcus aureus* i drugi sojevi) -- 3. dio: Izolacija i MPN postupak za male brojeve

Detekcija stafilokoknih enterotoksina tipa SEA do SEE (do danas poznato više od 20 stafilokoknih enterotoksina) :

- HRN EN ISO 19020:2017 Horizontalna metoda za imunoenzimsko dokazivanje prisutnosti stafilokoknih enterotoksina u hrani !!!!



EURL CPS
European Union Reference Laboratory for
Coagulase Positive Staphylococci

Laboratory of food safety

SBCL Unit,
Staphylococcus team

Circular letter addressed to:
• the Heads of the National Reference
Laboratories of the European Union for
Coagulase Positive Staphylococci;

Maisons-Alfort, 26th September 2025

Subject: Temporary Exemption from the VIDAS SET2 Kit
Instructions for Use – Freezing of Sample Extracts

Dear colleagues,

File followed by:
Yacine NIA
Isabelle MUTEL

Direct line:
+33 1 49 77 27 56

E-mail address:
eurl-staphylococci@anses.fr

Ref.:
CPS-Cr-202512L

As part of its annual European-wide Proficiency Testing (PT) program for National Reference Laboratories (NRLs), the EURL for CPS ensures compliance with regulatory standards for the detection of staphylococcal enterotoxins. In accordance with the EN ISO 19020 standard, the use of commercial kits VIDAS® SET2 (bioMérieux) and RIDASCREEN® SET Total (R-Biopharm) is mandated.

During the implementation assessments of this standard by NRLs using the VIDAS® SET2 kit, a discrepancy was identified between the sample extract storage recommendations outlined in EN ISO 19020 (§8.6) and those specified in the technical instructions provided by bioMérieux.

To address this issue, the EURL for CPS participated in two complementary studies evaluating the stability of staphylococcal enterotoxins types SEA to SEE in food extracts stored at -20°C ± 5°C:

- A preliminary study conducted in collaboration with the Irish NRL.
- A comprehensive study carried out in partnership with bioMérieux.

The results confirmed the stability of the five enterotoxins (SEA to SEE) in extracts from five food matrix categories (milk and dairy products, meat products, fish products, ready-to-eat meals and desserts) after 45 days of freezing. An extended study up to 180 days is currently underway.



anses

Based on these findings, bioMérieux is considering revising the technical instructions of the VIDAS® SET2 kit to authorize extract freezing and enable delayed analysis starting in 2026.

Temporary Exemption Granted

As an exceptional measure, the EURL for CPS hereby authorizes NRLs to deviate from the current instructions of the VIDAS® SET2 kit and freeze sample extracts for up to **45 days post-extraction**. This exemption is also applicable to the Proficiency Testing exercise scheduled for March 2026.

This temporary exemption granted is validated until the publication of the new version of technical instructions by bioMérieux

For further information, please contact: eurl-staphylococci@anses.fr

Yacine NIA
EURL for CPS Manager deputy

Izvještaj EFSA-e i ECDC-a o zoonozama za 2023. godinu

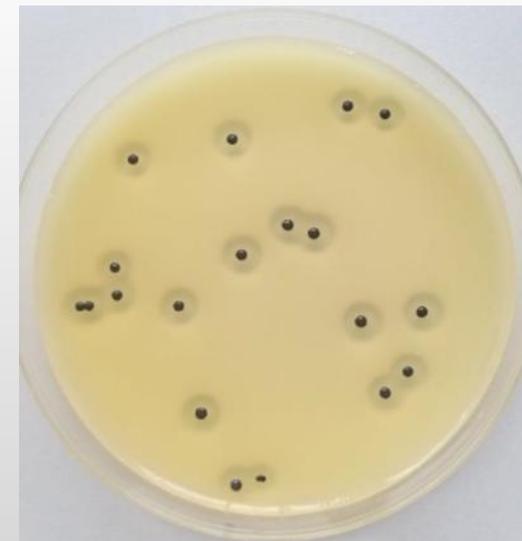
Pet država članica (Hrvatska, Njemačka, Grčka, Italija i Španjolska) dostavile su podatke o prisutnosti *Staphylococcus spp.* u hrani.

Analizirano: **6700 uzoraka hrane**

Pozitivni nalazi: **5,4%**

Najčešće pozitivne kategorije hrane:

- sirovo mlijeko
- ostali prerađeni proizvodi i pripremljena jela
- mesni proizvodi spremni za konzumaciju
- različite vrste sireva (svježi, tvrdi, meki, polutvrđi)
- maslac



Izvještaj EFSA-e i ECDC-a o zoonozama za 2023. godinu



STAFILOKOKNI ENTEROTOKSINI

- **U skladu s Uredbom (EZ) br. 2073/2005**
- Sedam država (Bugarska, Grčka, Italija, Rumunjska, Slovenija, Slovačka i Španjolska) prijavilo je podatke o enterotoksinima
- Distribucijska razina
Pozitivno: 5/2557 uzoraka (0,2%) – svi u Španjolskoj
Pozitivna kategorija: tvrdi sirevi od ovčjeg mlijeka
- Proizvodna razina
Pozitivno: 8/1076 uzoraka (0,7%) – u Italiji i Rumunjskoj
Pozitivne kategorije: meki i polutvrđi sirevi od kravljeg mlijeka, sirevi od miješanog mlijeka (krava, ovca, koza)
- **Podaci izvan okvira Uredbe (EZ) br. 2073/2005**
- Dvanaest država članica prijavilo je dodatne rezultate
Pozitivno: 20/ 5316 (0,38%)
Pozitivne kategorije: ostali prerađeni proizvodi i gotova jela

SFPO – Interlaboratory study on the Staphylococcal Food Poisoning Outbreak Investigaion (svibanj, 2024.)



- Uredba EZ 2073/2005 definira SE kriterij **samo za sir i mliječne proizvode.**
- **EFSA podaci:** rizik postoji i u **miješanoj hrani i mesnim proizvodima**

Godišnja radionica 2022.:

- NRL-ovi traže **vježbu za testiranje sposobnosti istraživanja epidemija**

Radni program 2023./2024.:

- NRL-ovi traže izazovnije vrste matriksa s kojima se mogu susresti tijekom rutinskih analiza SFPO-a u njihovim laboratorijima
- Pripremljen scenarij epidemije uzrokovane kontaminacijom hrane stafilokoknim enterotoksinima i poslan sudionicima zajedno s uzorcima.
- Scenarij kreiran uzimajući u obzir različite kontaminante (**CPS i enterotoksine**) i različite matrikse



2.1 SFPO SCENARIO

The EURL for CPS has prepared the following scenario and sent it to participants with samples :

Letter addressed to: National Reference Laboratory for CPS

Maisons-Alfort, 23th of April 2024.

During the Paris 2024 Olympic Games, three restaurants located in an Olympic village serves 500 meals for spectators during lunch. Spectators enjoyed this event until 22nd of April where a large food poisoning outbreak was declared. The competent authority decided to send some samples to the national reference laboratory for analysis and explained the situation in the email below:

"Dear NRL Colleague,

The manager of the Olympic village alerted the competent authority as well as the emergency services after the appearance of symptoms of a gastric epidemic on 22nd of April 2024. First investigation showed that 80 people present symptoms of vomiting with abdominal pain and diarrhea. 5 children and 2 elderly people were hospitalized. Symptoms appeared between 1 and 3 hours after having lunch in the restaurant "La Belle Etoile".

The next step of the investigation showed that this restaurant has received 100 people including the 80 sick people. Over the 80 ill, 47 consumed only Apple juice, Cheese with bread and mousse of chocolate, and 33 took Paella and mousse of chocolate and water. No information was available on the meal consumed by the 20 people who are not sick.

Responsible of the restaurant explained that the cheese was purchased recently from a local producer. However, paella and mousse of chocolate were prepared in the restaurant by two different persons.

Symptoms and time of onset suggest that staphylococcal enterotoxins may be responsible of this food borne outbreak. Thus, we decided to send to your NRL 4 samples for analysis. 30 mL of apple juice, 50 g of cheese, 50 g of paella and 50 g of mousse of chocolate were sampled and sent to your laboratory in refrigerated conditions.

Results expected from your NRL should help the competent authority to elucidate the source of the food borne outbreak.

End of the letter sent to participants"

consumed by 47 people

| Menu 1 | |
|--------|----------------------|
| ✓ | Apple juice, |
| ✓ | Cheese with bread |
| ✓ | Mousse of chocolate, |

consumed by 33 people

| Menu 2 | |
|--------|---------------------|
| ✓ | Water |
| ✓ | Paella |
| ✓ | Mousse of chocolate |

Figure 1. menu consumed by people having symptoms



SFPO – Interlaboratory study on the Staphylococcal Food Poisoning Outbreak Investigation



- Izazov oko izbora metode, kvalitete podataka, učinkovitosti odabranih metoda te sposobnosti identificiranja izvora trovanja
- Broj laboratorija koji su sudjelovali: 17
- Matriks (50g): Sok od jabuke
Paella
Sir
Čokoladni mousse
- Primjenjene metode: Određivanje broja koagulaza pozitivnih stafilokoka (HRN EN ISO 6888-1:2021)
Dokaz stafilokoknog enterotoksina imunoenzimatskom metodom (ISO 19020:2017)
WGS
- Očekivani rezultati:

Table 1. Codification and expected results

| Codification | Matrix | CPS enumeration (cfu/ml or cfu/g) | SE detection (EN ISO 19020) | In-house ELISA method | CPS species | se genes |
|--------------|------------------|------------------------------------|-----------------------------|-----------------------|-----------------|----------------------|
| M1 | Apple Juice | Not contaminated | Not detected | Not detected | / | / |
| M2 | Paëlla | 10 ⁶ to 10 ⁸ | Detected | Detected: SEA | <i>S.aureus</i> | <i>nuc, sea, sex</i> |
| M3 | Cheese | Not contaminated | Detected | Detected: SED | <i>S.aureus</i> | |
| M4 | Chocolate mousse | 10 ⁶ to 10 ⁸ | Not detected | Not detected | / | <i>nuc, selW</i> |

Table 5. Results obtained by participants for the matrix M1 (Apple juice)

| Lab code | CPS Enumeration (cfu/ml) | SE detection (EN ISO 19020) | In-house ELISA method | CPS species | se genes | Typing MLST |
|------------------|--------------------------|-----------------------------|-----------------------|--------------|--------------|-------------|
| Expected results | CPS not enumerated | Not detected | Not detected | Not detected | Not detected | / |
| 16 | < 10 | Not detected | / | / | / | / |

Table 6. Results obtained by the participants for the matrix M2 (Paëlla)

| Lab code | CPS Enumeration (cfu/g) | SE detection (EN ISO 19020) | In-house ELISA method | CPS species | se genes | Typing MLST |
|------------------|------------------------------------|-----------------------------|-----------------------|----------------------------|----------------------|-------------|
| Expected results | 10 ⁶ to 10 ⁸ | detected | SEA | <i>S. aureus</i> | <i>nuc, sea, sex</i> | / |
| 16 | 1.5x10 ⁷ | Detected | / | <i>S.aureus ssp aureus</i> | / | / |

Table 7. Results obtained by the participants for the matrix M3 (Cheese)

| Lab code | CPS Enumeration (cfu/g) | SE detection (EN ISO 19020) | In-house ELISA method | CPS species | se genes | Typing MLST |
|------------------|-------------------------|-----------------------------|-----------------------|--------------|--------------|-------------|
| Expected results | CPS not enumerated | Detected | SED | Not detected | Not detected | / |
| 16 | < 10 | Detected | / | / | / | / |

Table 8. Results obtained by participants for the matrix M4 (Chocolate mousse)

| Lab code | CPS Enumeration (cfu/g) | SE detection (EN ISO 19020) | In-house ELISA method | CPS species | se genes | Typing MLST |
|------------------|------------------------------------|-----------------------------|-----------------------|----------------------------|------------------|-------------|
| Expected results | 10 ⁶ to 10 ⁸ | Not detected | / | <i>S. aureus</i> | <i>nuc, selW</i> | / |
| 16 | 3.0x10 ⁷ | Not detected | / | <i>S.aureus ssp aureus</i> | / | / |

Uspješno provedeno!

PT CPS (lipanj, 2024.)



- Broj laboratorija koji su sudjelovali: 34
- Matriks (23g): Kozice
- Primjenjene metode: HRN EN ISO 6888-1 i/ili HRN EN ISO 6888-2

ILPT REPORT # Anses_LSAI_24_01_EURL_CPS_enumeration

Appendix 6. Individual z'-score for level 1 and z-score for level 2, by participants

| Lab code | LEVEL-1/6888-1 | LEVEL-1/6888-2 | LEVEL-2/6888-1 | LEVEL-2/6888-2 |
|----------|----------------|----------------|----------------|----------------|
| Lab_1 | -0,3 | 0,0 | -1,0 | -1,1 |
| Lab_2 | | 0,0 | | 0,4 |
| Lab_3 | 0,0 | 0,5 | 0,5 | 0,9 |
| Lab_4 | | -0,3 | | 0,0 |
| Lab_5 | | -0,7 | | -0,3 |
| Lab_6 | 1,4 | | 0,4 | |
| Lab_7 | -0,2 | 0,5 | 0,0 | 0,2 |
| Lab_8 | | 0,0 | | 0,5 |
| Lab_9 | | 1,0 | | -9,8 |
| Lab_10 | 0,0 | | -0,3 | |
| Lab_11 | | -0,3 | | -0,1 |
| Lab_12 | | 0,3 | | 0,6 |
| Lab_13 | -2,8 | | -1,4 | |
| Lab_14 | | -0,9 | | -0,7 |
| Lab_15 | | 0,7 | | -1,5 |
| Lab_16 | -0,5 | 0,5 | 0,4 | 1,2 |
| Lab_17 | -0,9 | | 0,2 | |
| Lab_18 | | -1,6 | | -1,1 |
| Lab_19 | | 2,8 | | 1,8 |
| Lab_20 | -0,5 | | 0,6 | |
| Lab_21 | | -0,2 | | 0,0 |
| Lab_22 | | 0,0 | | 0,5 |
| Lab_23 | 1,9 | | 2,2 | |
| Lab_24 | 0,7 | 0,0 | 1,4 | 0,8 |
| Lab_25 | -3,8 | | -6,1 | |
| Lab_26 | -0,9 | | -0,5 | |
| Lab_28 | | -0,7 | | 0,0 |
| Lab_29 | | -1,0 | | 0,4 |
| Lab_30 | | -0,2 | | -0,7 |
| Lab_31 | | -1,4 | | |
| Lab_32 | | 6,5 | | 0,0 |
| Lab_33 | 3,2 | | 2,9 | |
| Lab_34 | -0,7 | | -1,2 | |
| Lab_35 | | 0,2 | | 0,2 |

End of the report

APPENDIX 4. Results obtained by EN ISO 6888 -1

| Lab Code | Blank samples | | Level 1 | | Level 2 | |
|----------|---------------|-------|-------------|---------------------------|-------------|---------------------------|
| | Sample Code | CFU/g | Sample Code | Log ₁₀ (CFU/g) | Sample Code | Log ₁₀ (CFU/g) |
| 1 | 179 | < 10 | 85 | 4,255272505 | 159 | 6 |
| 3 | 144 | < 10 | 64 | 4,301029996 | 105 | 6,255272505 |
| 6 | 157 | < 10 | 1 | 4,491361694 | 82 | 6,230448921 |
| 7 | 163 | < 10 | 137 | 4,278753601 | 46 | 6,176091259 |
| 10 | 171 | < 10 | 103 | 4,301029996 | 67 | 6,113943352 |
| 13 | 8 | < 10 | 125 | 3,929418926 | 95 | 5,924279286 |
| 16 | 2 | < 100 | 104 | 4,230448921 | 10 | 6,230448921 |
| 17 | 78 | < 100 | 109 | 4,176091259 | 98 | 6,204119983 |
| 20 | 55 | < 100 | 84 | 4,230448921 | 52 | 6,278753601 |
| 23 | 175 | < 10 | 180 | 4,556302501 | 154 | 6,556302501 |
| 24 | 158 | < 10 | 106 | 4,397940009 | 18 | 6,414973348 |
| 25 | 122 | < 10 | 114 | 3,792391689 | 187 | 5,113943352 |
| 26 | 13 | < 10 | 162 | 4,176091259 | 75 | 6,079181246 |
| 33 | 72 | < 100 | 99 | 4,72427587 | 102 | 7,193124598 |
| 34 | 54 | < 100 | 152 | 4,204119983 | 24 | 6,662757832 |

APPENDIX 5. Results obtained by EN ISO 6888 -2

| Lab Code | Blank samples | | Level 1 | | Level 2 | |
|----------|---------------|-------|-------------|---------------------------|-------------|---------------------------|
| | Sample Code | CFU/g | Sample Code | Log ₁₀ (CFU/g) | Sample Code | Log ₁₀ (CFU/g) |
| 1 | 179 | <10 | 85 | 4,301029996 | 159 | 5,982271233 |
| 2 | 30 | <10 | 169 | 4,301029996 | 74 | 6,230448921 |
| 3 | 144 | <10 | 64 | 4,361727836 | 105 | 6,322219295 |
| 4 | 83 | <10 | 174 | 4,255272505 | 51 | 6,176091259 |
| 5 | 21 | <10 | 94 | 4,204119983 | 86 | 6,20573931 |
| 7 | 163 | <10 | 137 | 4,361727836 | 46 | 6,204119983 |
| 8 | 141 | 0 | 133 | 4,301029996 | 57 | 6,255272505 |
| 9 | 173 | <10 | 34 | 4,431363764 | 131 | 4,477121255 |
| 11 | 160 | <10 | 178 | 4,255272505 | 138 | 6,146128036 |
| 12 | 25 | <10 | 56 | 4,342422681 | 185 | 6,278753601 |
| 14 | 50 | <10 | 12 | 4,176091259 | 26 | 6,041392685 |
| 15 | 7 | <10 | 113 | 4,397940009 | 87 | 5,913813852 |
| 16 | 2 | <10 | 104 | 4,361727836 | 10 | 6,380211242 |
| 18 | 110 | <10 | 70 | 4,079181246 | 153 | 5,977723605 |
| 19 | 166 | <10 | 60 | 4,672097858 | 89 | 6,477121255 |
| 21 | 16 | <10 | 32 | 4,278753601 | 29 | 6,176091259 |
| 22 | 79 | <10 | 183 | 4,301029996 | 93 | 6,255272505 |
| 24 | 158 | <10 | 106 | 4,301029996 | 18 | 6,301029996 |
| 28 | 132 | <10 | 19 | 4,204119983 | 22 | 6,176091259 |
| 29 | 24 | <10 | 41 | 4,161368002 | 76 | 6,230448921 |
| 30 | 88 | <10 | 5 | 4,278753601 | 11 | 6,041392685 |
| 31 | 142 | <10 | 130 | 4,113943352 | 31 | > 5,477712125 |
| 32 | 184 | <10 | 23 | 5,176091259 | 146 | 6,176091259 |
| 35 | 33 | <10 | 121 | 4,322219295 | 128 | 6,204119983 |

Table 1. Test portion description

| Level | Targeted contamination (CFU.g ⁻¹) | Test portion / lab | Test portion (g) | Effective contamination achieved (CFU.g ⁻¹) |
|------------------|---|--------------------|------------------|---|
| Negative (blank) | <10; <100 | 1 | 23 | <10 |
| 1 | 1,0 x 10 ³ - 1,2 x 10 ⁴ | 1 | 23 | 1,3 x 10 ⁴ |
| 2 | 1,0 x 10 ⁵ - 1,2 x 10 ⁶ | 1 | 23 | 9,1 x 10 ⁵ |

Uspješno provedeno!

- PT CPS 2025. u tijeku



Table 2: Mean values and RSD obtained after homogeneity test using Vidas SET 2 and Ridascreen SET total

| Spiking level | Matrix | Vidas SET 2 | | Ridascreen SET total | |
|---------------|-----------------|-----------------|---------|----------------------|---------|
| | | Mean value (TV) | RSD (%) | Mean value (AU) | RSD (%) |
| 0 | Tabbouleh | Not detected | / | Not detected | / |
| 1 | Whole milk | 0.56 | 8.8 | 0.46 | 14.5 |
| 2 | Cantal (cheese) | 1.41 | 9.9 | 0.94 | 10.0 |



PT SA TOX (travanj, 2025.)

Table 1 : Codification of samples received and analyzed by participants

| Matrices | Tabbouleh | Whole milk | Whole milk | Whole milk | Cantal | Decoy samples | |
|----------------------|-----------|------------|------------|------------|--------|---------------|---------------------|
| Contamination levels | 0 | 1 | 1 | 1 | 2 | | |
| Number of replicates | 1 | 3 | 3 | 3 | 1 | | |
| NRL code | | | | | | Code | Matrix/level |
| 1 | 341 | 27 | 264 | 165 | 320 | 35 | Milk / level 1 |
| 2 | 102 | 277 | 6 | 100 | 115 | 41 | Milk / level 1 |
| 3 | 157 | 220 | 83 | 99 | 214 | 160 | Milk / level 1 |
| 4 | 76 | 110 | 111 | 152 | 275 | 190 | Milk / level 1 |
| 5 | 296 | 31 | 12 | 7 | 218 | 69 | Milk / level 1 |
| 6 | 149 | 119 | 71 | 207 | 241 | 16 | Milk / level 1 |
| 7 | 9 | 56 | 44 | 86 | 84 | 216 | Milk / level 1 |
| 8 | 196 | 174 | 171 | 252 | 15 | 177 | Milk / level 1 |
| 9 | 298 | 227 | 306 | 14 | 74 | 166 | Milk / level 1 |
| 10 | 79 | 217 | 108 | 162 | 70 | 276 | Milk / level 1 |
| 11 | 24 | 191 | 159 | 139 | 51 | 310 | Milk / level 1 |
| 12 | 129 | 199 | 182 | 45 | 43 | 109 | Tabbouleh / level 0 |
| 13 | 67 | 331 | 304 | 273 | 313 | 5 | Tabbouleh / level 0 |
| 14 | 26 | 223 | 113 | 75 | 294 | 94 | Tabbouleh / level 0 |
| 15 | 322 | 224 | 186 | 236 | 34 | 247 | Tabbouleh / level 0 |
| 16 | 292 | 50 | 303 | 20 | 123 | 39 | Tabbouleh / level 0 |
| 17 | 61 | 309 | 48* | 187 | 8 | 259 | Tabbouleh / level 0 |
| 18 | 150 | 29 | 210 | 77 | 154 | 238 | Tabbouleh / level 0 |
| 19 | 151 | 95 | 147 | 104 | 167 | 173 | Tabbouleh / level 0 |
| 20 | 185 | 125 | 328 | 3 | 97 | 38 | Tabbouleh / level 0 |
| 21 | 73 | 251 | 230 | 36 | 135 | 137 | Tabbouleh / level 0 |
| 22 | 293 | 280 | 286 | 197 | 66 | 170 | Cheese / level 2 |
| 23 | 311 | 266 | 2 | 317 | 141 | 288 | Cheese / level 2 |
| 24 | 232 | 90 | 47 | 333 | 124 | 58 | Cheese / level 2 |
| 25 | 300 | 332 | 106 | 326 | 316 | 270 | Cheese / level 2 |
| 26 | 260 | 81 | 103 | 49 | 112 | 146 | Cheese / level 2 |
| 27 | 21 | 302 | 284 | 180 | 228 | 145 | Cheese / level 2 |
| 28 | 202 | 291 | 87 | 305 | 172 | 203 | Cheese / level 2 |
| 29 | 37 | 338 | 299 | 222 | 336 | 158 | Cheese / level 2 |
| 30 | 92 | 40 | 278 | 140 | 258 | 237 | Cheese / level 2 |
| 31 | 148 | 105 | 321 | 318 | 168 | 343 | Cheese / level 2 |
| 32 | 281 | 1 | 17 | 42 | 19 | 96 | Cheese / level 2 |

- Broj laboratorija koji su sudjelovali: 32
- Matriks: CANTAL SIR
SALATA
MLIJEKO
- Primjenjene metode: Dokaz stafilokoknog enterotoksina imunoenzimatskom metodom (ISO 19020:2017)

➤ **Results lab 01**
Detection by Vidas SET 2 kit: 1010654530

| Matrix | Sample number | Intermediate value | Positive threshold | Result | Conclusion | Deviation |
|-------------------|---------------|--------------------|--------------------|--------------|--------------|-----------|
| Tabbouleh level 0 | 341 | 0.00 | 0.13 | Not detected | Satisfactory | No |
| | 27 | 0.28 | 0.13 | Detected | Satisfactory | No |
| Milk level 1 | 264 | 0.30 | 0.13 | Detected | Satisfactory | No |
| | 165 | 0.30 | 0.13 | Detected | Satisfactory | No |
| Cheese level 2 | 320 | 1.02 | 0.13 | Detected | Satisfactory | No |

Specificity: 100%, sensitivity: 100%, accuracy: 100%

Results of the Lab 01 for the ILPT are **satisfactory**.

Uspješno provedeno!



19. radionica EURL/NRL-ova za CPS –ANSES-Laboratorij za sigurnost hrane (01. i 02. travnja, 2025.)

- **PT organizacija:**

CPS: 2025. i 2027.

SE-ovi: 2025., 2026. i 2027.

SFPO vježba: 2026.

- **Smjernice i SOP-ovi**

Guidance document on the characterization of samples issued from a suspected Staphylococcal Food Poisoning Outbreak (SFPO)- DRAFT

- **Analitički razvoj**

LC-MS/MS metoda za detekciju SE-ova u hrani

Metode karakterizacije i tipizacije CPS-a

Razvoj SEs CRM-a **Development of SEs CRM as the SEA one is almost sold out !**

19. radionica EURL/NRL-ova za CPS –ANSES-Laboratorij za sigurnost hrane (01. i 02. travnja, 2025.)

EURL CPS kvartalno objavljuje Newsletter



| Food vehicle | |
|---|--|
| Category | Subcategory |
| Milk and <u>milk products</u> | <u>Cheese</u> |
| | <u>Milk</u> |
| | <u>Dairy products (other than cheeses)</u> |
| Meat and meat products (and their products) | <u>Poultry meat</u> |
| | <u>Meat and meat products, unspecified</u> |
| | <u>Pigmeat</u> |
| | <u>Bovine meat</u> |
| | <u>Sheep meat</u> |
| Fish and <u>fishery products</u> | <u>Crustaceans, shellfish, molluscs and their products</u> |
| | <u>Fish and fishery products</u> |
| <u>Mixed food</u> | <u>Mixed food</u> |
| <u>Buffet meals</u> | <u>Buffet meals</u> |
| <u>Bakery products</u> | <u>Bakery products</u> |
| <u>Eggs and egg products</u> | <u>Eggs and egg products</u> |
| Food of non-animal origin | <u>Confections</u> |
| | <u>Fruits (and juices)</u> |
| | <u>Herbs and spices</u> |
| <u>Other foods</u> | <u>Vegetables (and juices)</u> |
| | <u>Canned food products</u> |
| | <u>Cereal products and legumes</u> |
| | <u>Other foods/Unspecified</u> |

| Type of setting | |
|--|--|
| Category | Subcategory |
| <u>Household</u> | <u>Household</u> |
| Restaurant, pub, street vendors, take away | <u>Restaurant or Cafe or Pub or Bar or Hotel or Catering service</u> |
| | <u>Mobile retailer or market/street vendor</u> |
| | <u>Take-away or fast-food outlet</u> |
| Canteen or Catering to Workplace, school, hospital | <u>School or kindergarten</u> |
| | <u>Residential institution (nursing home or prison or boarding school)</u> |
| | <u>Canteen or workplace catering</u> |
| | <u>Hospital or medical care facility</u> |
| <u>Other settings</u> | <u>Others</u> |
| | <u>Multiple places of exposure in one country</u> |
| | <u>Camp or picnic</u> |
| <u>Unknown</u> | <u>Farm</u> |
| | <u>Multiple places of exposure in more than one country</u> |

Review of Staphylococcal Food Poisoning Outbreaks and scientific valorization of EURL for CPS network

Period: January 2024 – April 2024

Number: 9

| SFPO information/results | |
|---|--|
| Country | |
| Date | |
| Suspected food | |
| Context | |
| Nb of cases | |
| Symptoms | |
| Time of onset | |
| CPS enumeration (EN ISO 6888-2) | |
| SE detection (EN ISO 19020) SEA to SEE | |
| se genes (Multiplex End Point PCR) 11 genes: sea, to see, seg, seb, sei, sei, sei, sei, sep. | |
| Genomic analysis (NAuRA) | |
| In-house ELISA (EURL CPS methods) SEA, SEB, SEC, SED, SEE, SEH, SEG and SEI | |



EURL CPS
European Union Reference Laboratory for
Coagulase Positive Staphylococci
<http://eurl-staphylococci.anses.fr>



**Guidance document on the
characterization of samples issued from a
suspected Staphylococcal Food Poisoning
Outbreak (SFPO) [choice 1]**

Or

**Recommendations for characterization of
samples issued from a suspected
Staphylococcal Food Poisoning Outbreak
(SFPO) [choice 2]**

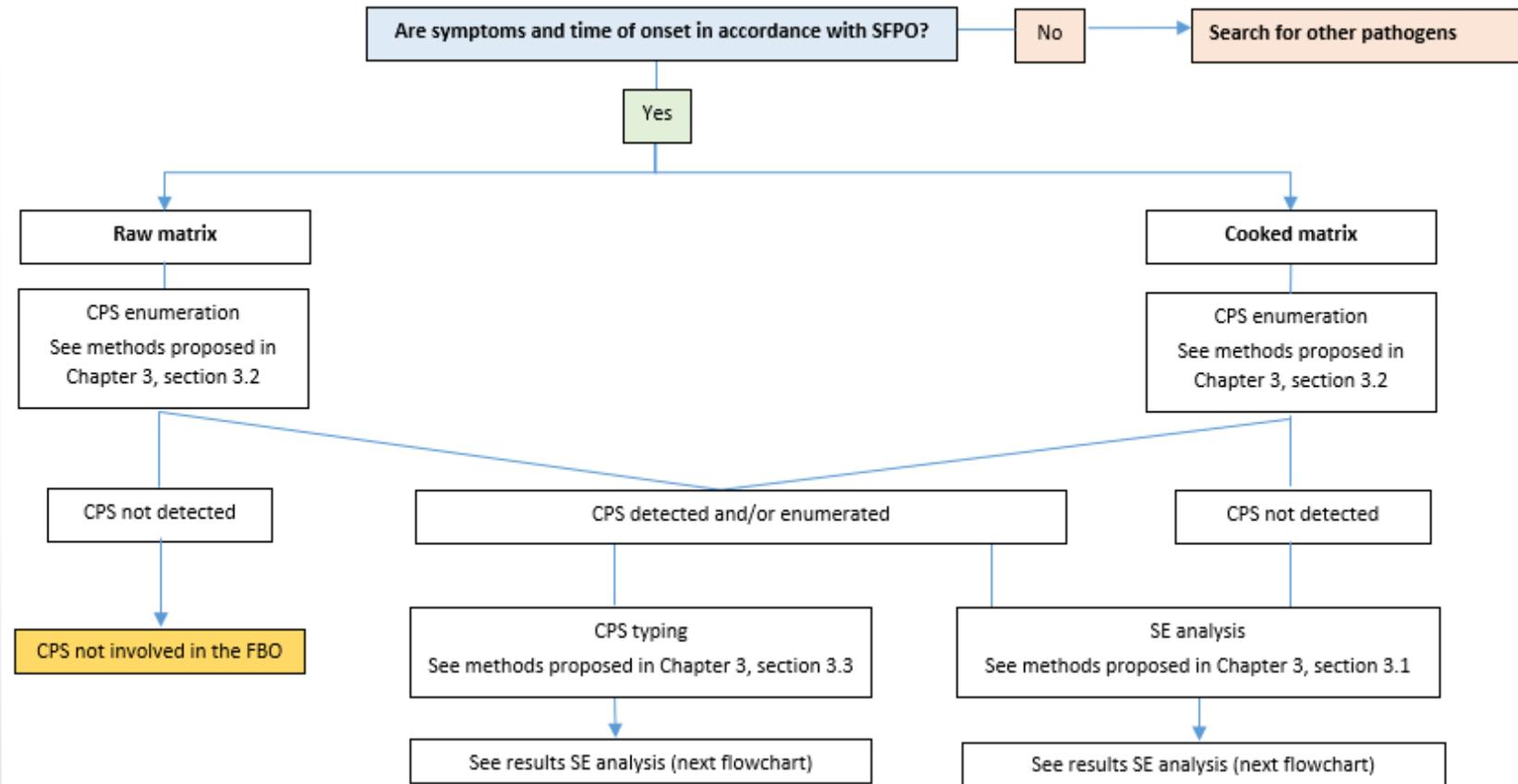
Vodič u izradi, komentari do
31.01.2026., pregled komentara do
kraja travnja 2026.

Kontekst i primjena Vodiča

U ovom dokumentu EURL predlaže:

- usklađeni postupak obrade uzoraka (zaprimanje, skladištenje i transport)
- analitički tijek koji treba primijeniti u laboratoriju kako bi se provele relevantne analize i interpretacija rezultata (epidemiološka istraga nije obuhvaćena ovim dokumentom)

Vodič služi kao smjernica NRL-ovima tijekom analize uzoraka povezanih sa stafilokoknim trovanjem hranom (SFPO)



Izvor: Guidance document on the characterization of samples issued from a suspected Staphylococcal Food Poisoning Outbreak (SFPO)

LC-MS/MS metoda za detekciju SE u hrani



Detection of SEs by Mass-spectrometry: progress of the validation study

Saïda Belarbi

EURL for CPS workshop

April 1st - 2nd, 2025



LC-MS/MS metoda za detekciju SE u hrani

Validacija LC MS/MS metode za detekciju 8 različitih tipova SE (SEA, SEB, SEC, SED, SEE, SEG, SEH i SEI) za 5 kategorija hrane (mliječni proizvodi, hrana spremna za konzumaciju, meso, riblji proizvodi, deserti)

Tijek pripreme uzoraka

1. Ekstrakcija SE toksina (EN ISO 19020)
2. Pročišćavanje i koncentracija
3. Enzimsko digestija
4. LC MS/MS analiza i obrada podataka

Izazov: nedostatak dostupnih standarda i/ili protokola za validaciju metoda za analizu bakterijskih toksina, a posebno stafilokoknih enterotoksina u prehrambenim matriksima !!!!!

Specifičnost: nema lažno pozitivnih rezultata, pouzdana komplementarna metoda uz ELISA-u

Osjetljivost: učinkovitost detekcije varira ovisno o kombinaciji (toksin/matriks)

ELISA (antigen \longleftrightarrow antitijelo)

- **Prednosti**
- Visoka osjetljivost (LOD = 0,02 do 0,15 ng/g)
- Brza, jednostavna za izvođenje, jeftina
- Kvalitativna i kvantitativna
- **✗ Ograničenja**
- Troši veliku količinu antitijela
- Mogući lažno pozitivni rezultati

Buduće aktivnosti EURL-a

- Revizija kriterija detekcije (ISO/SC9 WG30)
- Optimizacija pročišćavanja uzoraka radi poboljšanja osjetljivosti (ako je potrebno)
- Studija obnovljivosti s različitim LC MS/MS instrumentima
- Izračun LOD-a za svaki SE toksin

Hvala na pažnji!



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