



OIV-OENO 632-2021 Analytical and microbiological control techniques analyses common to all monographs

THE GENERAL ASSEMBLY,

IN VIEW OF the Article 2, paragraph 2 b) iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL of the “Microbiology” Expert group,

CONSIDERING the need to update the analytical and microbiological control techniques,

DECIDES to reorganise and modify paragraphs 1, 2, 3, 4, 5, 6 and 7 of file COEI-2-CONBAC in the *International Oenological Codex* as follows (file COEI-2-CONBAC contains many pages, however just the first 7 pages appear in this draft resolution because the modifications to be made only concern these first 7 pages of the file):

1. Preliminary rehydration of yeasts (*Saccharomyces* and non-*Saccharomyces*): ADY (Active Dry Yeasts), AFY (Active Frozen Yeasts), COY (Compressed Yeasts), CRY (Cream Yeast Preparations), ENY (Encapsulated [beads] or Immobilised Yeasts), “levain de tirage” (yeast starter for tirage)

Weigh approx. 10 g of the preparation under sterile conditions (note the exact weight for final calculation of the concentration).

Adjust to 100 mL with sterile peptone saline water* under sterile conditions at 20-37 °C or according to the manufacturer’s recommendations.

Slowly homogenise using a rod, a stomacher technique or a magnetic stirrer for 5 min.

Stop stirring and allow to stand for 20 min at room temperature of between 20-30 °C.

Homogenise again at room temperature for 5 min.

Under sterile conditions, prepare serial decimal dilutions in water or sterile peptone saline water* and proceed with microbiological controls on the homogenised stock solution.

In the case of “levains de tirage” used for sparkling wines, sample 1 mL under sterile conditions, prepare serial decimal dilutions in water or sterile peptone saline water* and proceed with microbiological controls on the homogenised stock solution

* Peptone salt solution: bacteriological peptone 1 g/L, sodium chloride 8.5 g/L, final pH 7.0

2. Preliminary rehydration of preparations of lactic acid bacteria

Weigh approx. 10 g of preparation of lactic acid bacteria under sterile conditions (note the exact weight for final calculation of the concentration).

Adjust to 100 mL with sterile peptone saline water* under sterile conditions (25°C).

Homogenise using a magnetic stirrer or a stomacher technique for 5 min.

Stop stirring and allow to stand for 20 min at room temperature of between 20-30 °C.

Homogenise again at room temperature for 5 min.

Under sterile conditions, prepare serial decimal dilutions using water or sterile peptone saline water* and proceed with microbiological controls.

* Peptone salt solution: bacteriological peptone 1 g/L, sodium chloride 8.5 g/L, final pH 7.0

3. Microbiological control of other products in the *International Oenological Codex*

(products for which the control of yeasts, bacteria and/or moulds is requested)

Weigh approx. 10 g of the oenological product to control under sterile conditions (note the exact weight for final calculation of the concentration).

Adjust to 100 mL with sterile peptone saline water* under sterile conditions.

Homogenise using a magnetic stirrer or a stomacher technique for 5 min.

Under sterile conditions, prepare serial decimal dilutions in water or sterile peptone saline water* and proceed with microbiological controls.

* Peptone salt solution: bacteriological peptone 1 g/L, sodium chloride 8.5 g/L, final pH 7.0

4. Enumeration of total yeasts

YM agar medium (MALT WICKERHAM)

| | |
|----------------------|------|
| Bacteriological agar | 15 g |
| Yeast extract | 3 g |
| Malt extract | 3 g |
| Peptone | 5 g |
| Glucose | 10 g |

Water q.s. 1000 mL

YPD

Yeast extract 10 g

Peptone 20 g

Glucose 20 g

Agar 10 g

Water q.s. 1000 mL

Just after preparation, autoclave the medium at 120 °C for 20 min.

In case of prolonged incubation time, add chloramphenicol to a 100 mg/L final concentration to prevent bacterial growth.

After inoculation with the appropriate dilutions of the sample in order to reach 30-300 colonies, incubate the dishes at 25-30°C under aerobic conditions for 48 to 72 hours.

Count the number of CFU in the dishes containing 30-300 colonies and refer to the weight of the dry matter.

In addition to the media proposed, any equivalent medium internationally recognised for growth of these microorganisms may be used.

5. Enumeration of non-*Saccharomyces* yeasts

5.1. Lysine medium

The yeasts are cultivated in the lysine medium whose composition is as follows:

Agar 20 g

L-lysine monohydrochloride 5 g

Glucose 1 g

Bromocresol purple 0.015 g

Water q.s. 1000 mL

Adjust to pH 6.8 ± 0.2

Bring to the boil for 1 min to ensure complete dissolution, then autoclave at 120°C for 20 min.

In case of prolonged incubation time, add chloramphenicol to a 100 mg/L final concentration to prevent bacterial growth.

After inoculation with the dilutions of the sample, the dishes are incubated at 25°C or 30°C for 48 to 96 hours.

Count the number of CFU (dishes of 30-300 colonies) and refer to the weight of the dry matter.

In addition to the media proposed, any equivalent medium internationally recognised for growth of these microorganisms may be used.

5.2. **YPD medium with addition of cycloheximide** at 10 mg/L, and incubation for 6-7 days under aerobic conditions

In case of prolonged incubation time, add chloramphenicol to a 100 mg/L final concentration to prevent bacterial growth.

6. Enumeration of viable lactic acid bacteria

Modified MRS (Man, Rogosa and Sharpe)

The bacteria are cultivated in a MRS medium (Man, Rogosa, Sharpe 1960), with addition of tomato juice, and the composition is as follows:

| | |
|---------------------|--------|
| Agar agar | 15 g |
| Bacto-peptone | 10 g |
| Meat extract | 8 g |
| Yeast extract | 4 g |
| Sodium acetate | 5 g |
| K_2HPO_4 | 2 g |
| Trisodium citrate | 2 g |
| $MgSO_4$ at 100mg/L | 2.5 mL |
| $MnSO_4$ at 20mg/L | 2 mL |



| | |
|-----------------------|-------------|
| Tween 80 | 1 mL |
| DL malic acid | 5 g |
| Tomato juice * | 200 mL |
| Glucose | 20 g |
| Or glucose + Fructose | 10 g+ 10 g |
| Adjust (HCl or NaOH) | q.s pH 4.8 |
| Distilled water | q.s 1000 mL |

(q.s.=quantity sufficient)

*Tomato juice is used to improve lactic bacterial growth. Preparation: take commercial (without additives) or homemade tomato juice, centrifuge at 4000g for 20 min, filter if necessary and use the clear juice.

Autoclave at 110°C for 20 min.

When pouring the medium into the Petri dish, add pimarcine to a 10 mg/L final concentration to inhibit the growth of yeast and mould.

Incubate at 25 °C under anaerobic conditions for 8 to 10 days.

In addition to the media proposed, any equivalent medium internationally recognised for growth of these microorganisms may be used.

7. Enumeration of mould

Czapeck-Dox/s agar medium

| | |
|--------------------------|-------|
| Agar agar | 15 g |
| Saccharose | 30 g |
| NaNO_3 | 3 g |
| K_2HPO_4 | 1 g |
| MgSO_4 | 0.5 g |



| | |
|-----------|--------------|
| KCl | 0.5 g |
| $FeSO_4$ | 0.01 g |
| Water | q.s. 1000 mL |
| Adjust to | pH 7 |

Autoclave at 120 °C for 20 min.

Add chloramphenicol directly to the medium in the Petri dish to a 100 mg/L final concentration to inhibit the growth of bacteria.

Incubate at 20 °C under aerobic conditions for 10 days.

In addition to the media proposed, any equivalent medium internationally recognised for growth of these microorganisms may be used.

8. Enumeration of acetic bacteria

| | |
|----------------------|--------------|
| Bacteriological agar | 20 g |
| Yeast extract | 5 g |
| Casein amino acids | 5 g |
| Glucose | 10 g |
| Adjust to | pH 4.5 |
| Water | q.s. 1000 mL |

Autoclave at 120°C for 20 min.

When pouring the medium into the Petri dish, add pimaricine to a 100 mg/L final concentration, to inhibit the growth of yeast and mould, and penicillin to a 12.5 mg/L final concentration to inhibit the growth of lactic acid bacteria.

Incubate at 25 °C under aerobic conditions for 4 days.

In addition to the media proposed, any equivalent medium internationally recognised for growth of these microorganisms may be used.