RESOLUTION OIV-OENO 632-2021

ANALYTICAL AND MICROBIOLOGICAL CONTROL TECHNIQUES ANALYSES COMMON TO ALL MONOGRAPHS

WARNING : This resolution amends the following resolutions :
- *OENO 17/2003*
- *OIV/OENO 328/2009*
- *OIV/OENO 329/2009*

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 concentation).

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.

Certified in conformity Paris meeting hybrid, 12th July 2021
The Director General of the OIV
Secretary of the General Assembly
Pau ROCA

© OIV 2021

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 concentation).

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

Certified in conformity Paris meeting hybrid, 12th July 2021
The Director General of the OIV
Secretary of the General Assembly
Pau ROCA

© OIV 2021

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.

Certified in conformity Paris meeting hybrid, 12th July 2021
The Director General of the OIV
Secretary of the General Assembly
Pau ROCA

© OIV 2021

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
Bromoscresol 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.

Certified in conformity Paris meeting hybrid, 12th July 2021
The Director General of the OIV
Secretary of the General Assembly
Pau ROCA

© OIV 2021

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

*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 pimaricine 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.

Certified in conformity Paris meeting hybrid, 12th July 2021
The Director General of the OIV
Secretary of the General Assembly
Pau ROCA

© OIV 2021

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

Certified in conformity Paris meeting hybrid, 12th July 2021
The Director General of the OIV
Secretary of the General Assembly
Pau ROCA

© OIV 2021

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.

Certified in conformity Paris meeting hybrid, 12th July 2021
The Director General of the OIV
Secretary of the General Assembly
Pau ROCA

© OIV 2021