CYTO-3D VINS

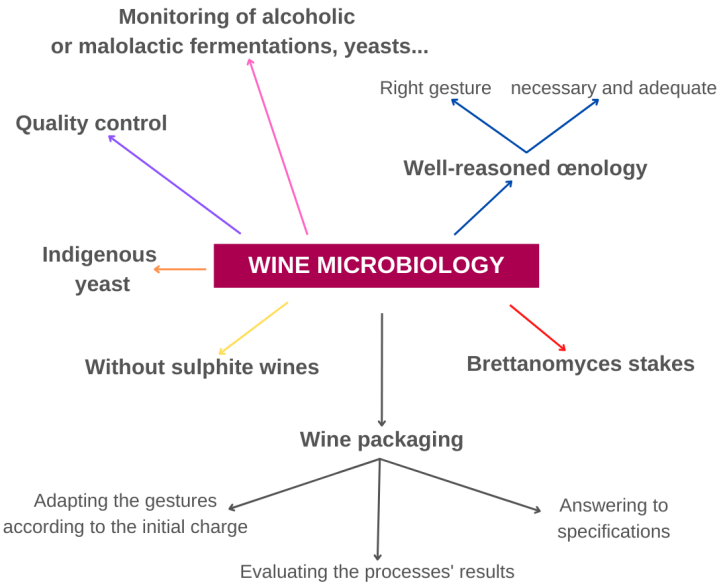
Understanding and mastering wine microbiology

HISTORY OF WINE MICROBIOLOGICAL ANALYSIS

From an analytical point of view, wine is a product of chemistry and biology. While there has been enormous progress in the productivity of chemical analysis in recent years, microbiology is still in the era of Pasteur, with its Petri dishes! Although other technologies have appeared more recently, such as PCR, these techniques are still suitable for research but not for routine analysis in terms of speed and competitiveness.

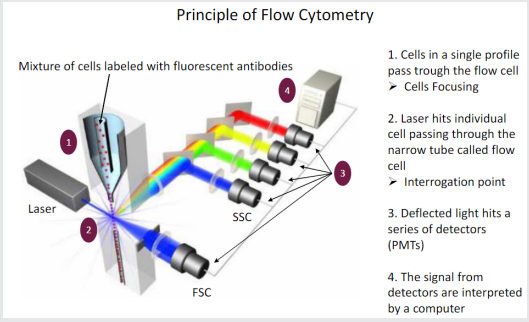
From harvest to packaging, however, there is a real interest in monitoring the microbiology of wine at various stages in the production process.

We therefore felt it was essential to develop a range of innovative and precise applications to finally make microbiological analysis a routine part of the process, in the same way as other physico-chemical parameters in wine.



FLOW CYTOMETRY

Flow cytometry is a technique for counting and measuring cell states. A first fluidic system allows cells suspended in a fluid to pass through a very fine groove, allowing these cells to be aligned one by one in front of one (or more) laser(s). Each cell then emits light signals that are analysed by an optical system: fluorescence emitted by the particle, refraction/obscuration of the light, structural size, etc. Based on these parameters, an electronic system interprets these signals and produces a cytogram, enabling the nature of the cells to be identified.



Flow cytometry is not a recent method: the first publications in oenology on the subject date back to 1994. But the limitations of the technique (sensitivity to background noise, high quantification limits, the need to concentrate samples and, above all, the difficulty of distinguishing between Saccharomyces and Brettanomyces) have reduced its use and spread in field oenology.

INNOVATIONS FROM THE DUBERNET LABORATORIES

Triple fluorescence-activated cell labelling (FACS technique)

The micro-organisms are exposed to fluorochromes (dyes with fluorescent properties). The fluorochromes absorb the dyes differently depending on their nature and physiological state. Unprecedented on this scale, this technique enables an original gating strategy to be used in conjunction with triple labelling, the discrimination of Brettanomyces and Saccharomyces yeasts in wines, and the quantification of yeasts and bacteria in a single analysis.

High-performance cytometers

The new cytometers are equipped with very high resolution detectors and high throughput. These latest-generation cytometers allow a high flow rate and are also equipped with several lasers, multiplying the amount of information carried on each particle. We are constantly investing in high-tech equipment: we recently acquired the Cytpix: a cytometer equipped with a camera and a fourth laser: these improvements enable us to be more precise in our perception of microbiota, particularly in the differentiation of lactic and acetic bacteria.

Practical sheet

CYTO-3D WINES

CONCRETE APPLICATION IN THE WINE CYCLE

With Cyto-3D vins, we offer you an innovative, exclusive and patented method capable of meeting the challenges of in the field. Microbiological analysis has become the essential tool for precise, preventive oenology:

- rapid results in less than 48 hours,
- control of all stages of wine production.

Evaluating the effectiveness of oenological techniques and treatments

Malolactic fermentation
CYTO-3D VINS
- control of total bacterial populations

Preparing the packaging
CYTO-3D VINS - VINS PREMIUM
- control of microbial populations before bottling/BIB
- quality control of filtration
- control of flash pasteurisation

Alcoholic fermentation
CYTO-MOUT if D > 1040
CYTO-3D VINS if D < 1040
- control of yeast implantation
- monitoring languishing fermentations

New or ageing wine
CYTO-3D VINS - VINS PREMIUM - BACT - EXPERTISE
- control of Brettanomyces and Saccharomyces
- control of total bacteria
- control of acetic and lactic bacteria

Packaged wine
CYTO-3D CONDITIONNE
- control of Brettanomyces and Saccharomyces
- control of total bacteria

OUR ANALYSIS MENUS

ANALYSIS CODE	PARAMETERS ANALYSED	SAMPLE REQUIRED	TIME
CYTO-MOÛT	**Total yeast :** vital living, VMI and dead **& bacteria** vital living	200 ml	48 h
CYTO-3D VINS *(package)* **CYTO-3D VINS PREMIUM** *(fee-for-service)*	**Brettanomyces** vital living*, VMI* and dead* **Saccharomyces** vital living*, VMI* and dead* **& total bacteria** vital living*, VMI and dead		
CYTO-3D BRETTS	**Brettanomyces** Vital living*, VMI* and dead* populations		
CYTO-3D BACT	**Lactic & acetic bacteria** vital living, VMI and dead populations		
CYTO-3D EXPERTISE	**Brettanomyces** vital living*, VMI* and dead* **Saccharomyces** vital living*, VMI* and dead* **& lactic & acetic bacteria** living vital, VMI and dead		
CYTO-3D PRISE DE MOUSSE	**Brettanomyces** living vital, VMI and dead **Saccharomyces** living vital, VMI and dead **& total bacteria** living vital, VMI and dead		
CYTO-3D CONDITIONNE	**Brettanomyces** vital living*, VMI* and dead* **Saccharomyces** vital living*, VMI* and dead* **& total bacteria** vital living*, VMI and dead	Packaged wine	

VMI Definition : Viable Metabolically Inactive (no esterase activity).
* analyses accredited to standard NF EN ISO 17025.

AN ACCREDITED METHOD

Since October 2021, Cyto-3D analyses have been carried out under COFRAC accreditation in accordance with the NF EN ISO 17025 standard.

Accreditation no. 1-0207
Scope available on www.cofrac.fr
Montredon-Corbières site

Accreditation no. 1-5833
Scope available on www.cofrac.fr
Orange website

cofrac ESSAIS

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CYTO-3D VINS

INTERPRETING CYTO-3D RESULTS

Cyto-3D vins provides direct and specific counts of the different microbial populations in wine, as well as their status:

CYTO-3D VINS

Saccharomyces Yeasts	Brettanomyces Yeasts	Total bacteria

VITAL LIVING CELLS
These are the living, metabolically active populations.
For example, in the case of brettanomyces, these are the populations that produce volatile phenols.

VMI CELLS (Viable Metabolically Inactive (absence of esterase activity))
These populations are not in a phase of multiplication, but constitute a form of latency. They can revitalise in response to a change in the wine's environment and represent a potential risk to the wine, but not an immediate one, unlike a vital living population.
These populations theoretically correspond to viable non-cultivable populations (VNC). They do not grow in Petri dishes.

DEAD CELLS
Dead cells present in the medium.
Cells theoretically counted in PCR, distorting the interpretation of the result.

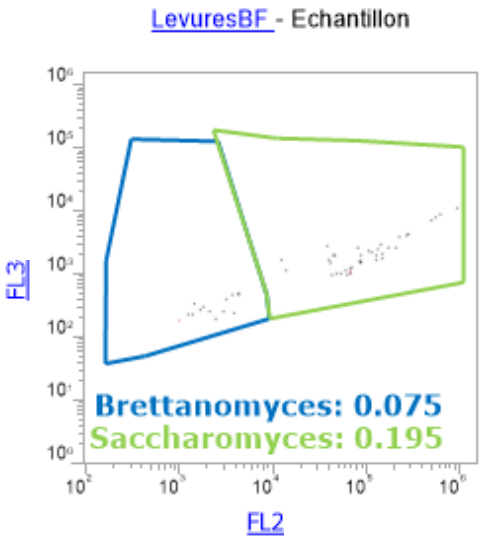
HOW TO READ THE ANALYSIS REPORT

By convention in microbiology, results are expressed in scientific writing, to make them easier to read.
For example, 2,5e+04 events/mL means 2,5.10^4 events/mL or 25 000 events/mL.

Meaning of the parameters shown on the report :

Type of micro-organism	Sacch = Saccharomyces Brett = Brettanomyces Bact = Bactéries totales
State of the micro-organism	VV = Living vital VMI = Viable Metabolically Inactive (absence of esterase activity)

nd = undetected (< at the detection limit)



Cytogram showing the separation of Brettanomyces and Saccharomyces yeasts along the FL2 axis.

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CYTO-3D VINS

INTERPRETATION CRITERIA FOR THE STUDY OF BRETTANOMYCES

By increasing our annual microbiological analyses from 5,000 to 30,000, flow cytometry has enabled us to build up a substantial database for studying Brettanomyces.

Brettanomyces are yeasts present in musts and wines. They have an enzyme pool capable of metabolising certain phenolic acids naturally present in wines (p-coumaric acid and caftaric acid) into malodorous volatile phenols (4-Ethyl-phenol and 4-Ethyl-gaiacol respectively). These volatile phenols give wine its typical 'animal' aromatic character, which is now regarded as a major drawback by most wine professionals and consumers.

An environmental yeast for the natural ecology of wines

90% of red wines analysed contain vital living Brettanomyces populations and/or VMI populations. The presence of Brettanomyces is natural and almost systematic, and these yeasts can develop at any stage in the life of the wine: they can be found in the vineyard and on winemaking equipment.

Some oenological practices increase the risk of Brettanomyces

Cold pre-fermentation maceration, long pre-fermentation lag periods, sluggish or stopped AF, traces of glucose/fructose at the end of AF, unsuitable wine hygiene, uncontrolled temperatures during ageing, low levels of active SO2, uncontrolled microbiological populations during packaging, etc.

Controlling Brettannomyces therefore involves monitoring the size of the populations, in particular that of the vital living cells capable of producing volatile phenols: a process made possible by the monthly Cyto-3D monitoring.

Our observations have enabled us to establish the interpretation thresholds shown below:

Security zone
Monitor for possible high levels of VMI.

Surveillance zone
No rapid evolution of volatile phenols.
Treatment to be considered before bottling.
Monitor the wine within 3 to 4 weeks.

Action zone
Changes in volatile phenols in the medium term.
Treatment to be carried out.

Emergency zone
Very short-term evolution.
Urgent treatment required.

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CYTO-3D VINS

INTERPRETATION CRITERIA FOR SACCHAROMYCES YEASTS

Counting and monitoring Saccharomyces yeasts is an important management tool at different stages in the life of the wine.

DURING WINEMAKING

- Evaluation of vital live Saccharomyces populations. Good alcoholic fermentations are obtained when populations rapidly reach 10^7 event/ml.
- Leaven checking before incorporation in the case of winemaking with indigenous yeasts.
- Monitoring the end of languishing fermentations and taking rapid action if necessary.

DURING AGEING

Monitoring populations.
During this period, it is possible to find Saccharomyces yeasts, which are generally in a latent state and of the order of 10^4 event/ml. Particular attention should be paid to sweetened wines or wines with residual sugars, which should never have populations > 10^4 event/ml. If this is the case, action is strongly recommended, to be adapted according to the population level (racking, sulphiting, pasteurisation, etc.).

INTERPRETATION CRITERIA FOR BACTERIA

Bacteria are part of the wine microbiota, and their rapid counting using the Cyto-3D method is of interest for the precise control of winemaking and ageing operations.

DURING WINEMAKING

- Monitoring malolactic fermentation. The vital live bacteria population is generally at least 10^6 events/ml to ensure that MLF runs smoothly.
- Diagnosis of a malo start in the event of sluggish alcoholic fermentation.

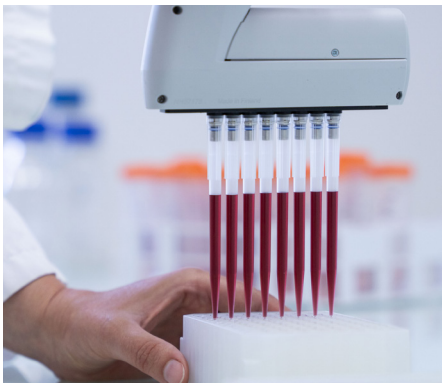
DURING AGEING

- Management of residual populations of vital living bacteria, particularly in the case of wines with no added sulphites.
- Anticipation of a rise in volatile levels by detecting an abnormal population during wine conservation. The populations usually found are of the order of 10^3 to 10^4 vital living bacteria, above 10^5 event/ml action is strongly recommended.

Practical sheet

CYTO-3D VINS

PREPARATION OF WINES FOR PACKAGING



Cyto-3D analyses are of particular interest in the management of pre-packaging microbiological populations, making it possible to finely control the wine preparation stages (racking, fining, pre-filtration, final filtration at bottling).
These are crucial because they help to reduce microbiological populations. It is important to remember that the filtration operations reduce microbiological populations by a factor of 100 to 1000, and are in no way sterilising operations.

This dynamic study, from the 'brut de cuve' wine to the 'pré-mise' wine, is a revolution in oenological analysis and a real working tool.

PACKAGED WINE

Cyto-3D analyses are carried out post-packaging. Their aim is to check the complete conformity of the product after packaging, with a complete microorganism balance being sought within a timeframe compatible with the needs of the industry.

The following criteria are used to check the packaging of dry wines:

Dry and sulphited red wines

Sacc VV (event/ml)	Brett VV (event/ml)	Sum of yeasts (sacch +brett) VMI (event/ml)	Bact VV (event/ml)	Bact VMI (event/ml)
< 10	< 10	< 200	< 10^3	< 10^4

Sulphited white and rosé wines

Sacc VV (event/ml)	Brett VV (event/ml)	Sum of yeasts (sacch +brett) VMI (event/ml)	Bact VV (event/ml)	Bact VMI (event/ml)
< 10	< 10	< 200	< 10^2	< 10^3

Wines containing residual sugars and sulphite-free wines

Sacc VV (event/ml)	Brett VV (event/ml)	Sum of yeasts (sacch +brett) VMI (event/ml)	Bact VV (event/ml)	Bact VMI (event/ml)
< 10	< 10	< 50	< 10^2	< 10^3

VV : «Vivantes Vitales», Living vital

VMI : Viable Metabolically Inactive (Absence of esterase activity)

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