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**Agroscope**

# Practical method for the detection of histamine- forming bacteria in milk

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FACEnetwork: Working Group «Technology» 28 February 2023

[www.agroscope.ch](http://www.agroscope.ch) | gutes Essen, gesunde Umwelt



# Discover unknown causes of known cheese defects: Too high content of histamine

- Cheese defect:
  - Trigeminal perception of "burning" and "pungent" during tasting
  - Undesirable formation of eyes, splits or cracks
  - Increased levels of histamine, which can lead to allergy-like symptoms in sensitive people, such as abdominal cramps, diarrhoea, flatulence, feeling of fever, reddening of the skin, rashes, itching, nausea or even vomiting
- Cause:
  - Continuous degradation of the amino acid histidine during ripening (splitting off the acid group)
- New finding from microbiome analysis:
  - *Lentilactobacillus parabuchneri* is responsible for the formation of histamine in cheese





## Recommendations in case of too high histamine content

- Persistent contamination in milking systems is the main cause
  - Check raw milk samples from each dairy farmer for the presence of histamine-forming bacteria (Simple practice method available)
    - Check cleaning parameters: too low cleaning temperatures? Too short cleaning period? Too low detergent dosage or use of unsuitable cleaning agents?
    - Processing environment control at the dairy farm concerned to identify the source: Milking unit? Milk line? Milk tank?
    - Careful cleaning or, if necessary, replacement (e.g. of seals)
  - Contaminated milk should be excluded from processing into raw milk products.



Deposits in the collection piece of the milking unit



# Emmental cheese declassified



**Histamine** 485 mg/kg  
Total biogenic amines 512 mg/kg



**Histamine** 721 mg/kg  
Total biogenic amines 774 mg/kg



**Histamine** 324 mg/kg  
Total biogenic amines 345 mg/kg



**Histamine** 577 mg/kg  
Total biogenic amines 624 mg/kg



# Implementation in cheese practice

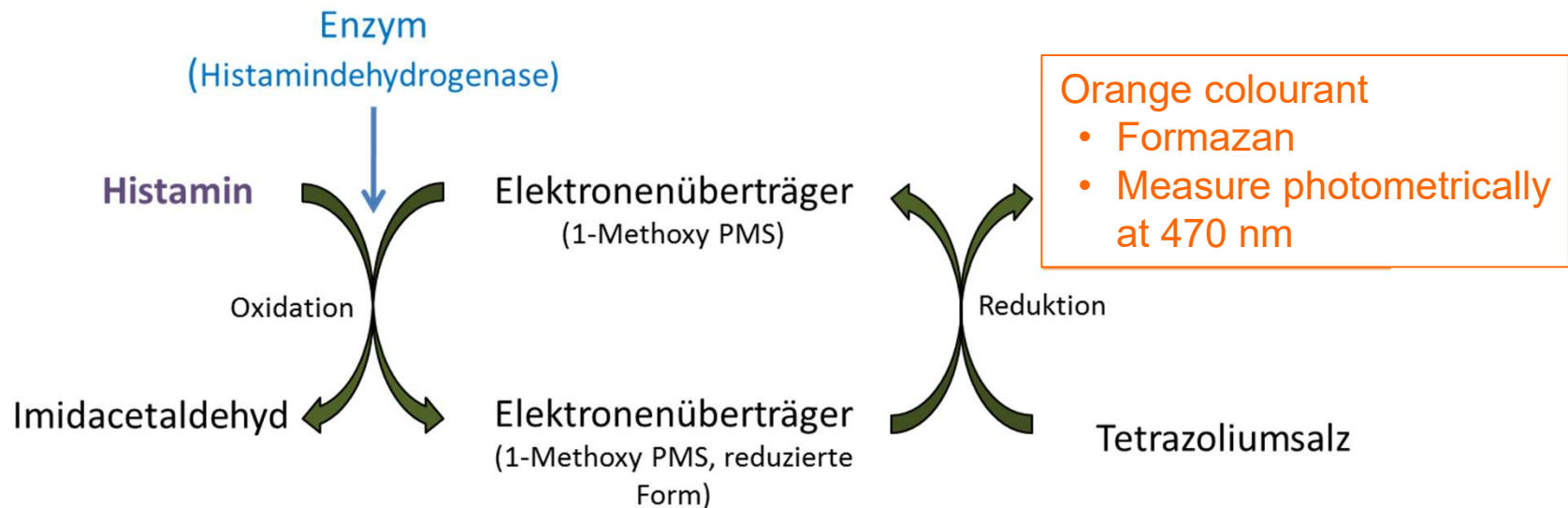
- Fact: Histamine formation in cheese is caused by *L. parabuchneri* in the vast majority of cases.
- Molecular biological detection of *L. parabuchneri* (using q-PCR) is very expensive and can only be done in a specialised laboratory.
- Solution: Practical method for the detection of histamine-forming bacteria in milk in routine laboratories.



# Method development for the detection of histamine formation

## Principle of the histamine test

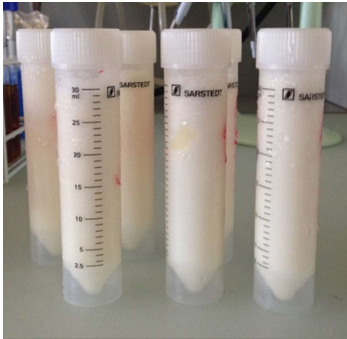
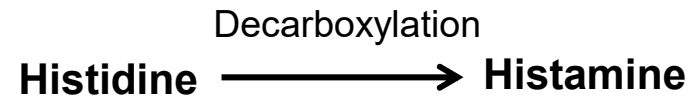
- Quantitative determination of histamine
- Enzymatic reaction



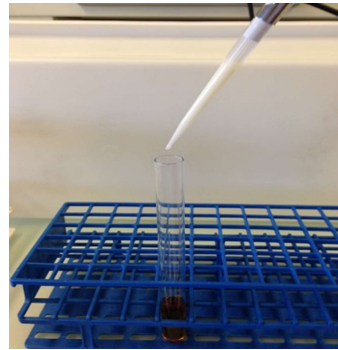


# Preparation and execution of the histamine test

## Preparation



Milk samples from farmer and/or cheesemaker



1. 1 ml milk in 5 ml broth (Melibiose + 0.3 % Histidine)



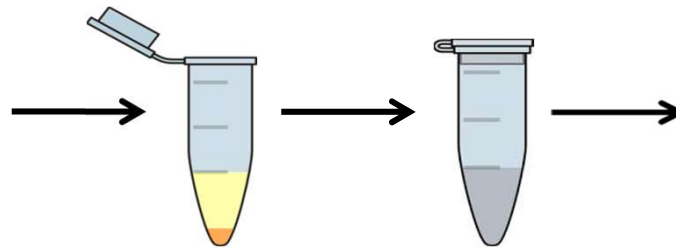
2. Incubation at 30 °C, Measurement of histamine after 4-7 days





# Preparation and execution of the histamine test

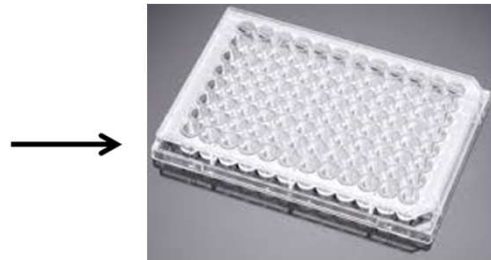
## Measurement



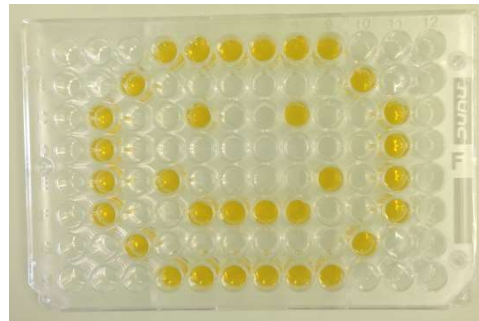
1. centrifuge 200  $\mu$ l

2. 10  $\mu$ l supernatant  
in 1 ml 0.1 M EDTA  
(pH 8)

3. 50  $\mu$ l solution +  
test components



4. Incubation 37 °C, 15  
min in microtitre plate



5. Positive milk samples

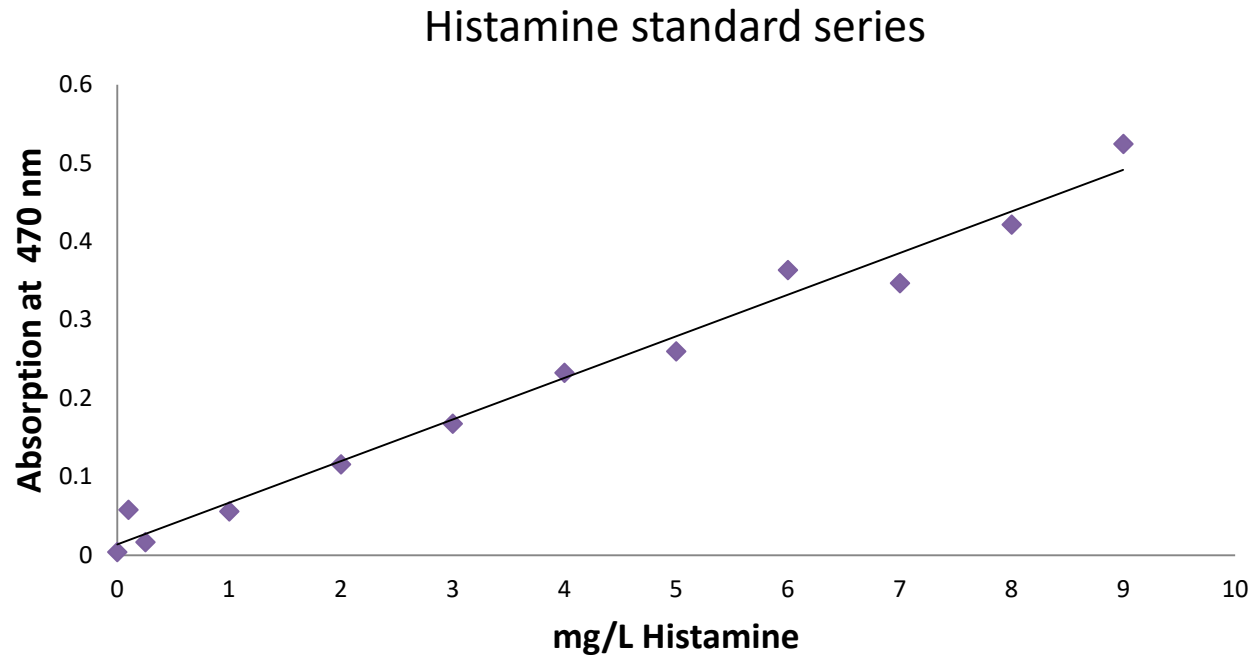


6. Measurement at 470 nm





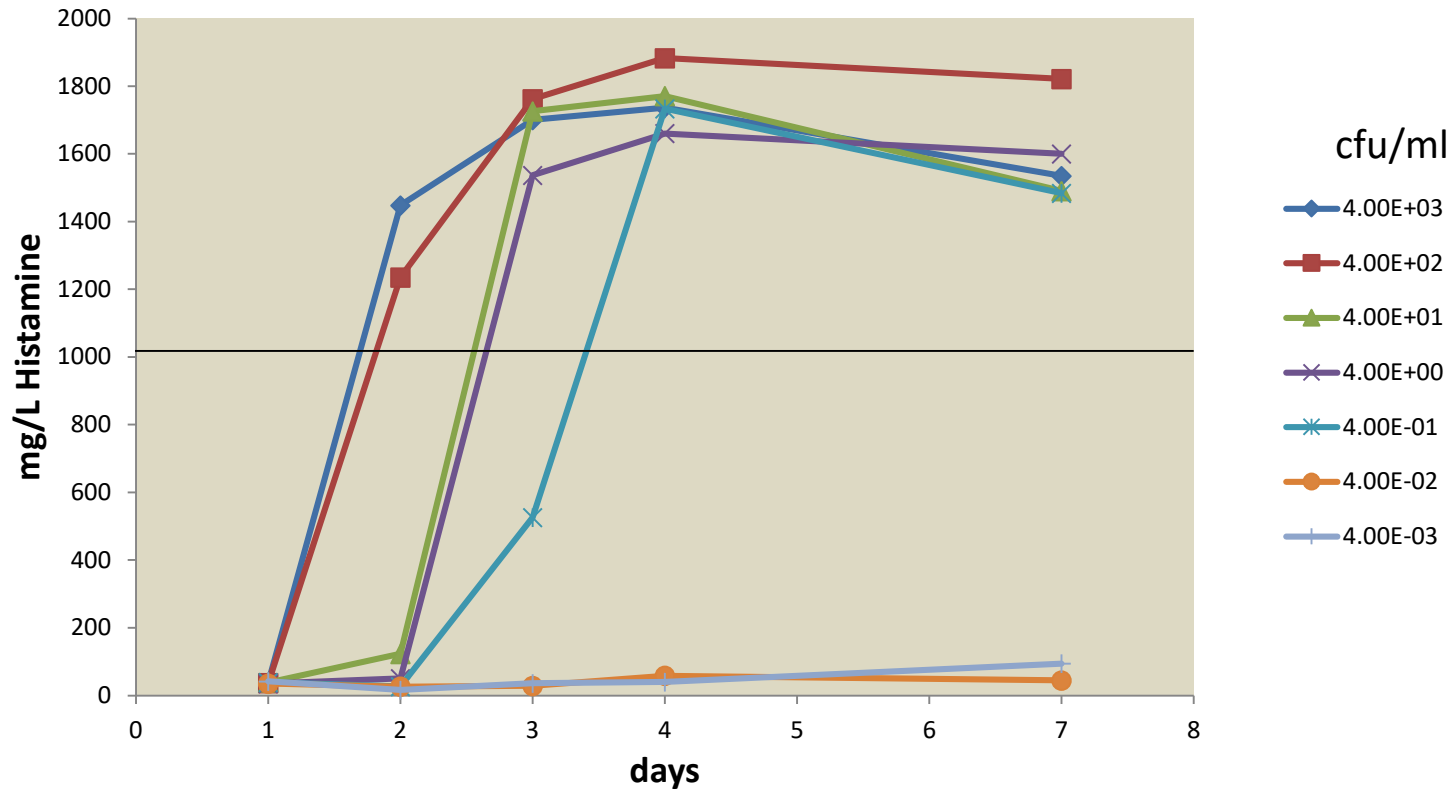
# Linearity



One considers a positive histamine value from an absorption of 0.1.



# Method for the detection of histamine-forming bacteria in milk



- Dilution series of *L. parabuchneri* ( $10^3$ -  $10^{-3}$  cfu/ml)



# Application in practice

Probe: Alpkäse  
7 Mte, Produktion 24.8.13, kleine Löchli, sandig, **brennend**, salzig, Aroma i.O.

Flüchtige Carbonsäuren total	mmol/kg	17.9
Ameisensäure	mmol/kg	0.5
Essigsäure	mmol/kg	<b>14.2 ↑</b>
Propionsäure	mmol/kg	0.0
i-Buttersäure	mmol/kg	0.2
n-Buttersäure	mmol/kg	2.3
Buttersäure berechnet aus Gärung	mmol/kg	1.4
i-Valeriansäure	mmol/kg	0.4
i-Caprinsäure	mmol/kg	0.0
n-Caprinsäure	mmol/kg	0.3
Kochsalz	g/kg	<b>21.6 ↑</b>
Histamin	mg/kg	<b>917 ↑↑</b>
Tyramin	mg/kg	13
Summe biogene Amine	mg/kg	<b>930 ↑↑</b>



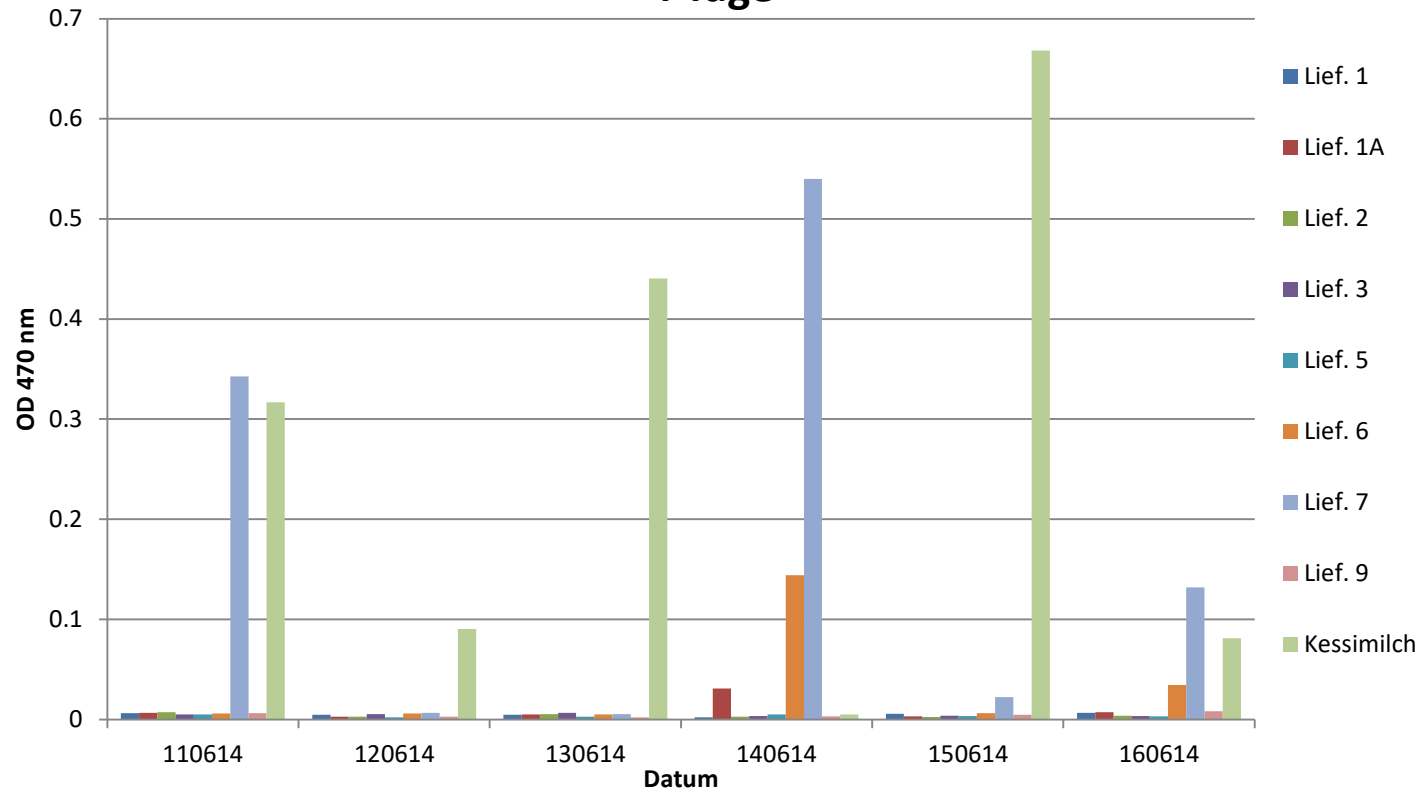
## What is the cause of the high histamine content?

⇒ Detection of histamine-forming bacteria in milk samples from farms and cheese dairy reveals sources of contamination



# Results

4 Tage



69

62

108

199

mmol/kg histamine in cheese  
3 months ripening

Interpretation: farmers 6, 7 and vat milk = **positive**



# Measures

## Farmer 6



- Clean the vessel for rinse water and empty it daily.



- Cleaning the outlet valve of the milk tank
- Do not place milk inlet pipe in tank on the floor



- Suspend the milking units at a greater distance from the ground

⇒ After implementation = 7 follow-up checks  
😊 **all histamine negative**



# Measures

## ▪ Farmer 7



- After cleaning by hand, additionally scald the milk dishes (at least 80°C).



- Replace air hoses and connect to the flushing unit for cleaning daily

- Cleaning of the milking machine (rinsing device), initial temperature 80°C, temperature at the end of cleaning at least 60°C (cleaning time 5-7 min.)

⇒ After implementation = 7 follow-up checks  
😊 **all histamine negative**



# Practical method for the detection of histamine-forming bacteria in milk

- More cost-effective than qPCR (35 CHF / sample)
- Semi-quantitative detection of contaminations up to 100 cfu/ml
- At least 4 days incubation time
- Only if required: Detection of *L. parabuchneri* by qPCR



## 2. ANALYSES MICROBIOLOGIQUES / MIKROBIOLOGISCHE ANALYSEN



N° article <i>Artikel-Nr.</i>	Description article/ <i>Beschreibung des Artikels</i>	Méthode <i>Methode</i>	Quantité/ <i>Menge</i>  (g ou/oder ml)	Délai/ <i>Zeitbed.</i>  (jours/ <i>Tage</i> )	Prix/Preis (CHF) <sup>x</sup>	
					sans TVA <i>ohne MWSt.</i>	TVA incl. <i>mit MWSt.</i>
MESL.533.0 <sup>P</sup>	<b>Bacillus cereus</b>	ISO	100 g/40 ml	1-2	32.50	35.00
MESL.524.0 <sup>na</sup>	<b>Bactéries lactiques / <i>Milchsäurebakt.</i></b>	LAAF / FALL	40 ml	4	48.00	51.70
MEEEX.136.0 <sup>na, pc</sup>	<b>Bactéries responsables de la formation d'<i>histamine</i> / <i>Histamin</i>bildende Bakterien</b>	Agroscope	10 ml	Sur demande/ auf Anfrage	35.00	37.70
MESL.003.0	<b>Cellules / <i>Zellen</i></b>	FIL/LAAF/FIL/FALL	250 ml	1	5.00	5.40
MESL.502.0 <sup>P</sup>	<b>E. Coli</b>	ISO	100 g	1	15.75	16.95
MESL.503.0	<b>E. Coli (eau) / <i>E. Coli (Wasser)</i></b>	ISO	250 ml	1	22.00	23.70
MESL.505.0 <sup>P G)</sup>	<b>Entérobactéries / <i>Enterobakterien</i></b>	ISO	100 g	1	15.75	16.95
MESL.506.0	<b>Entérocoques (eau) / <i>Enterokokken (Wasser)</i></b>	ISO	250 ml	2	22.00	23.70
MESL.501.0 <sup>P</sup>	<b>Germes aérobies mésophiles / <i>Aerobe mesophile Keime</i></b>	ISO	100 g/40 ml	3	15.75	16.95
	<b>Germes aérobies mésophiles (eau) /</b>					



# Thank you for your attention

