

2011

# MicroCheck User Guide

## Frequently Asked Questions

The following document provides answers to the most frequently asked questions concerning microbiology tests offered by National Milk Laboratories.



Microbiology Unit  
National Milk Laboratories  
March 2011





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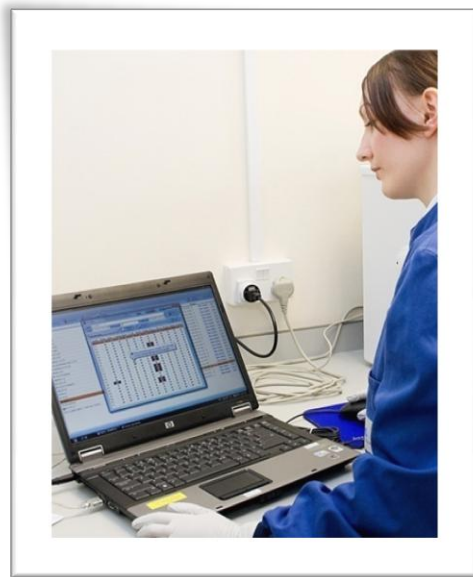
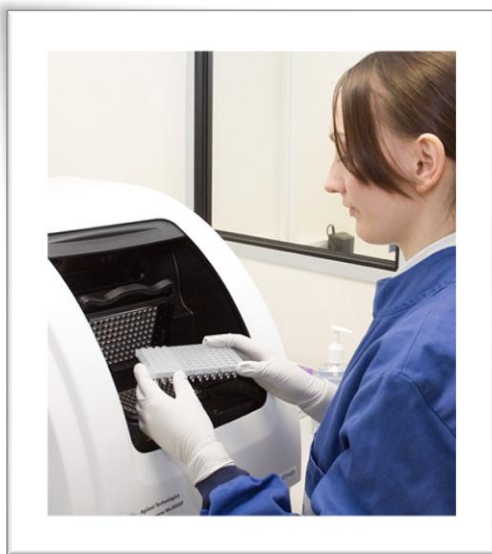
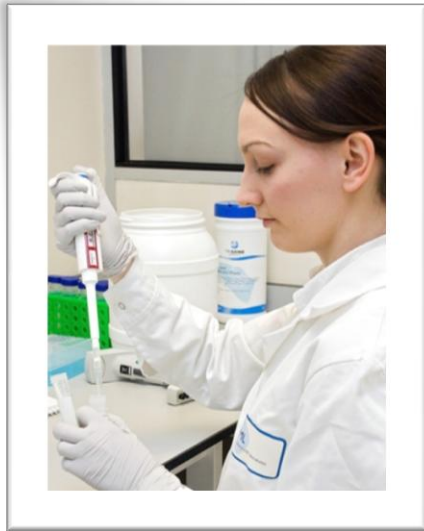
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### Customer Services Contact Details

Customer Services on 0844 7255567

[customerservices@nrm.co.uk](mailto:customerservices@nrm.co.uk)

## Introduction

### MicroCheck products

NML is committed to supplying a high quality and efficient testing service for its clients. Milk buyers, farmers and vets have been routinely using NMLs MicroCheck services since 2004. NMLs services include milk quality testing, antibiotic contaminant testing, traditional microbiology and modern molecular biology. NML have worked hard to ensure that customers can obtain results with ease in a timely manner.

This guide is intended to provide answers to common questions regarding NMLs Microbiology services. These services include:

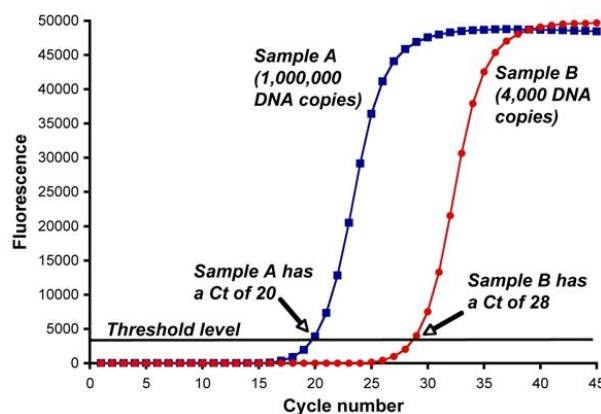
- qPCR
- Basic somatic cell counts (SCC)
- Culture testing

Each of these will be addressed in turn. It is important to note that SCCs are included within the Bacto Breakdown and Mastitis ID test suites.

## Test methods

### What is qPCR testing?

Quantitative Polymerase Chain Reaction (qPCR) is a molecular biological method for amplifying a specific DNA sequence within a sample. The reaction involves a DNA-binding fluorescent dye and requires a cyclical heating and cooling program. As specific DNA is formed, the fluorescence (proportional to the amount of DNA accumulating) is measured after each heat/cool cycle. If more DNA was present at the start, it takes a lower number of cycles for the amount of DNA to cross a threshold (Ct value). This method shows whether DNA from a pathogen was present and by how much.



### How does qPCR compare to bacterial culture for pathogen identification?

Both qPCR and culture are useful for mastitic pathogen detection and both have relative 'pros and cons'. The main advantage to qPCR is the ability to use bronopol preserved milk to prevent growth in transit and to enable room temperature transport. It is important to remember that DNA from both live and dead cells will be detected by qPCR whereas culture would only produce results for viable cells. If culture is used and no growths occur (in over 20% of cases), it can be useful to qPCR test the sample to see if pathogens can be detected. Furthermore, whilst qPCR does not detect as many potential mastitic pathogens as culture methods, qPCR is able to detect pathogens responsible for over 95% of the UK's mastitis-causing pathogens. Culture methods are still useful for further investigation or further confirmatory testing especially in cases where *Bacillus*, yeasts, *Pseudomonas* or fungi are suspected.

### What is a somatic cell count?

Somatic cells are 'bacteria-fighting' cells or white blood cells that move to an infection site e.g. udder, and minimize the infection. It can be indicative of sub-clinical mastitis i.e. where cows exhibit no outward signs of mastitis.

SCC is important for identifying the problem in the first instance to indicate that a mastitis ID needs to be performed, and so then the specific bug (bacteria) can be identified. The test can be carried out on both bulk milk samples and individual cow samples. Kits are available and preservative can be used if no culture testing is required.

### Why do I have a negative result for all the bacteria but a high somatic cell count?

Test Suite	Reason
<b>Bacto Breakdown Plant Hygiene (bacteriology)</b>	Has the sample been frozen? If so, the pathogens may have been killed and are no longer viable for our culture methods. Freezing can cause cell lysis and prevent growth for some species.
<b>Mastitis ID</b>	The cause may be a rare mastitic pathogen like algal sp., Yeast sp. or <i>Bacillus</i> sp. These are not identified by the NML test.

If clinical mastitis cases occur and milk is discarded, you will not identify potential causes for the clinical infection from bulk milk samples.

### Why don't I have a SCC reading?

If it states "Sour Milk Unreadable" for a mastitis sample then either the milk was already clotted upon leaving the cow or the preservative pill was not mixed thoroughly enough in the sample to prevent souring. If there are lumps in the milk it cannot pass through the fine tubing to obtain a somatic cell count reading.

If there is high bacterial content the milk may sour more rapidly regardless of the storage temperature. Psychrotrophs can grow at refrigeration temperatures and therefore if present can still cause the milk to sour. There have been examples of milk that has reached NML within 24 hours of sampling that has still been sour. For mastitis ID testing, always use the preservative provided.

### Why does it say “Insufficient Sample Size”?

Insufficient sample size means that there was not enough milk provided or that the storage sample was also used for a variety of other tests. Payment tests for the milk buyers will take priority over any other tests, including MicroCheck.

### I have always used bacterial culture, do you still offer this?

Yes we do, to an extent. Within our Bacto Breakdown service we culture for total viable counts (all live bacteria within the sample), Psychrotrophs (bacteria that can grow at refrigerated temperatures), Coliforms (indicative of faecal contamination) and Thermotolerants (bacteria that can withstand pasteurization at 63.5 °C).

## Test Types

### What is the difference between a Bacto Breakdown and a Mastitis ID?

Bacto Breakdowns utilise traditional bacteriology in addition to qPCR. They are performed on bulk tank samples and provide information concerning Plant (farm) hygiene and also provide an indication of what mastitic pathogen DNA is present at the herd level. Mastitis ID can either be performed on a bulk tank sample or be performed on individual cow samples. Remember that milk from clinical cases is usually discarded and thus bulk tank qPCR will not identify specific clinical causes in these cases. It is simply the identification of pathogens through qPCR. The following table details the tests included within each test suite:

Individual Test	Bacto Breakdown	Mastitis ID	Parlour Hygiene
<b>Total Viable Count (TVC) is a measure of all bacteria that is “alive” in the milk sample</b>	✓		✓
<b>Coliforms are indicative of faecal (environmental contamination)</b>	✓		✓
<b>Thermotolerants are bacteria that can withstand pasteurization at 63.5C</b>	✓		✓
<b>Psychrotrophs are bacteria that are able to grow at refrigerated temperatures</b>	✓		✓
<b>Mastitis ID for 11 key mastitis causing pathogens along with Staphylococcus spp penicillin resistance using qPCR</b>	✓	✓	

### What are the costs for testing (veterinary pricelists are available)?

Test Suite	Price
Ad-hoc Bacto Breakdown (Bulk only)	£45 + VAT per sample
Quarterly Surveillance Bacto Breakdown (4 per year tested automatically using NML sample)	£150 + VAT per year
1-5 Mastitis IDs ordered in one go	£17.50 + VAT per sample
>5 Mastitis IDs ordered in one go	£15 + VAT per sample
Parlour Hygiene Suite (Bulk only)	£20 + VAT per sample

### Do you offer antibiotic sensitivity on the bacterial targets identified?

We do not offer antibiotic sensitivity testing; however we do identify the *Staphylococcus* penicillin-resistant gene,  $\beta$ -lactamase.

## Sample Condition

### How can samples be sent in?

Test Suite	Condition
Bacto Breakdown or Parlour Hygiene	For '7-day collected' customers, NML-stored payment backup samples can be used. For non-7-day clients, please contact your milk buyer regarding collection of an additional sample.
Mastitis ID	Fresh, frozen or bronopol preserved. Ensure preservative is mixed thoroughly and if the sample already has clots a SCC result will not be obtained.

## Results

### In what form can I receive my results?

- Post
- Fax
- Email
- Herd Companion website - Micro Monitor (Passwords are available from NMR)

### Why did I not receive my qPCR results the same day the sample was received?

It is stated that we aim to return results the same day, but this is not a guaranteed service for the following reasons:

- DNA is extracted from the samples the same day, but only so many samples can fit into the machine, therefore samples received first are tested first.
- The sample may have an Internal Amplification Control (IAC) failure. IAC failures are caused by large quantities of bacterial DNA competing and inhibiting the reaction. The test is repeated with diluted DNA and the result calculated to account for the dilution used.

### **I have ordered a Bacto Breakdown, but I have only received one report, why?**

Bacto Breakdowns generate 2 reports:

- qPCR report sent as soon as the testing is completed, usually within 24 hours
- Plant hygiene culture report within 5 working days

### **How do I obtain my Herd Companion username and password?**

As an NMR/NML customer, you will have an assigned username and password. If you give your details to customer services, they will be able to issue you with your access details. Please note that if the Parlour Hygiene suite is tested alone (not with the Bacto Breakdown suite), results are not yet available via Herd Companion.

### **Can my vet access my results on Herd Companion?**

If farmers sign an access agreement form, their vet will be able to instantly access health and testing reports. We recommend that farmers ensure their vet is informed of their test results and Herd Companion is a quick and easy way of doing this.

### **How do I know when results have arrived?**

If we have an email address for the farmer, an automated email will be sent to ensure the farmer (and the vet - see above) is informed that results are available via Herd Companion.

## **Interpretation**

### **qPCR**

#### **What do I do about this particular pathogen?**

Customers are advised to consult their vet regarding result interpretation. It is important to remember that it isn't always the most abundant organism that is the cause of the infection. Furthermore, the result is only as good as the sample. If strict aseptic technique is applied to sampling, you can be more confident of the results.

Refer to Mastitis ID guidance notes (attached in the appendix). Bulk milk results should not be used as a guide for treating individual cows. Remember that clinically infected milk will not contribute to the bulk tank. Also, consider the timing of infection for individual cows. This could provide an indication as to whether the pathogen is associated with dry period infection or whether it has been picked up during lactation. Consult NMR records in conjunction with your vet.

#### **Can I still have a qPCR test if the cow is being treated with antibiotics?**

Milk from cows being treated with antibiotics can be tested; however the test will not distinguish between living and dead bacteria. This is useful if you wish to determine, which pathogen is present to ensure the correct antibiotic is being used, but it can take at least two weeks before dead bacteria are flushed out.

## Plant Hygiene

### **The number of bacteria for Thermoturics, Psychrotrophs and Coliforms doesn't add up to the Total Viable Count (TVC), why not?**

The results depend on the spread of the bacteria within the milk as it is shared between the tests. There may also be additional organisms making up the TVC that are not covered by thermoturics, psychrotrophs and coliforms.

### **Why do I have a high Coliform/ Thermoturic/ Psychrotroph/ TVC? What do I do?**

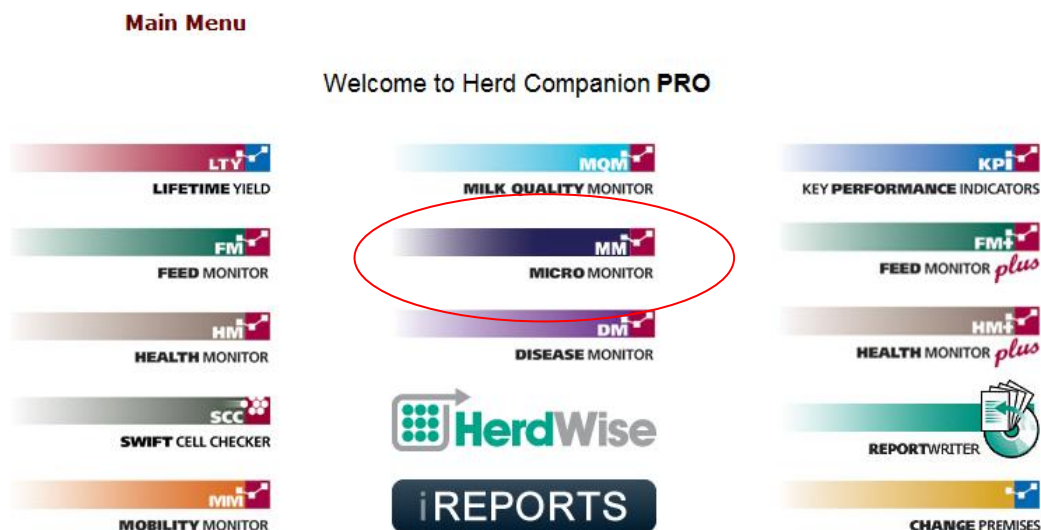
Refer to the Bacto Breakdown guidance notes (attached in the appendix).

National Milk Laboratories can only advise customers to speak to milk hygiene consultants or veterinary practitioners.

## Appendix 1 – Online reporting

### How do I access results online?

Both farmers and vets can access all test results via the Herd Companion website (<http://www.nmr.co.uk/herd-companion/>). When you access the main menu, click on the Micro Monitor (for Microbiology results).



Within Micro Monitor you can select the report you wish to view (see figure below).

**Welcome to Micro Monitor**

High somatic cell count and bactoscan results can have a major impact on your milk cheque, not just in terms of payment penalties but also lost production and poor animal health.

The MICROCHECK service will help you take the guess work out of milk quality management.

Our testing is carried out using cutting edge PCR technology which identifies the DNA of mastitis causing pathogens. In addition, the laboratory uses traditional culture methods to identify bacteria which cause issues in the bulk tank or milking plant.

Results can be reported by fax, post or email as well as through the Micro Monitor site. Mastitis results using PCR technology are usually available within 24 hours of the sample reaching the laboratory. Culture results are available within five working days. Results can be viewed by your veterinary surgeon for their detailed interpretation and you should then seek their advice on the best course of action to take

Bacto Breakdown Suite	Mastitis ID Suite	NMR Mastitis Tracker
<p>The Bacto Breakdown suite detects plant hygiene pathogens and mastitis causing pathogens in bulk tank milk samples</p>	<p>Mastitis ID is a test suite which detects mastitis causing pathogens in individual cow or quarter milk samples</p>	<p>NMR Mastitis tracker uses monthly NMR recording samples to provide information on pathogens present in selected groups of cows including persistent high</p>

If you select 'Mastitis ID suite', you are given a list of test reports (displayed below).

### Mastitis ID Reports

Below is a list of Mastitis ID tests which have been conducted, click on the report column to view a chosen report

Batch Number	Sample Number	Sample Date	Report
80012536B	1766	02/09/2010	Mastitis ID
80012535B	3170	02/09/2010	Mastitis ID
80012535B	4	02/09/2010	Mastitis ID
80012536B	7563	02/09/2010	Mastitis ID
80012535B	7587	02/09/2010	Mastitis ID
80012536B	7616	02/09/2010	Mastitis ID
80012535B	7691	02/09/2010	Mastitis ID
80012536B	88	02/09/2010	Mastitis ID
80012535B	8871	02/09/2010	Mastitis ID
80012536B	9044	02/09/2010	Mastitis ID

### Individual Cow Testing

To use the Mastitis ID service you need to order a sample testing kit. Once samples have been taken these can be returned to the lab in the pre-paid packaging supplied. To order a Mastitis ID kit please call 'NMR Customer Services' on 0844 7255567.

If you click on any of the reports, it will display the test results for that individual animal. An example report is displayed below.

### NML Mastitis ID Report

<b>Herd Number</b>		<b>Sample Type</b>	Individual			
<b>Producer ID</b>		<b>Sample Date</b>	02/09/2010			
<b>Batch Number</b>		<b>Test Date</b>	06/09/2010			
<b>Sample ID</b>		<b>Test Location</b>	NML – Wolves			
Somatic Cell Count (SCC)		18,000				
Pathogen type	Pathogen	Test Result	Prevalence Level			Proportion
			+	++	+++	
Contagious	<i>Staphylococcus aureus</i>	NEG				
	<i>Staphylococcus species (CNS)</i>	POS				
	<i>Streptococcus agalactiae</i>	NEG				
	<i>Streptococcus dysgalactiae</i>	NEG				
	<i>Corynebacterium bovis</i>	NEG				
Environmental	<i>Streptococcus uberis</i>	NEG				
	<i>Escherichia coli</i>	NEG				
	<i>Enterococcus species</i>	NEG				
	<i>Klebsiella species</i>	NEG				
	<i>Serratia marcescens</i>	NEG				
	<i>A. pyogenes and P. indolicus</i>	NEG				
Penicillin resistance	<i>Staphylococcal <math>\beta</math> – lactamase gene</i>	NEG				

## Appendix 2 – Individual Cow Mastitis ID guidance notes

### qPCR individual cow test result interpretation

This test is designed to detect the presence of 11 key mastitis pathogens (responsible for >95% of cases) along with penicillin resistance in *Staphylococcus* species.

Results are reported according to the level of bacteria detected in preserved milk. Bacterial growth in transit does not occur but multiple pathogens can be detected.

#### Result Interpretation – Mastitis Pathogens

Result	Low	Medium	High	Interpretation
NEG				Negative/not detected
POS				Positive in low numbers
POS				Positive in moderate numbers
POS				Positive in high numbers

Where >90% or >99% of the bacterial load is attributed to one pathogen, this will appear in the report.

Pathogens can be split roughly into two groups (though some can fit into both groups):

- 1) Contagious mastitis pathogens: *Staphylococcus aureus*, other *Staphylococcal* species, *Corynebacterium bovis*, *Streptococcus agalactiae* & *Streptococcus dysgalactiae*.

Abbreviated hereafter as **C**.

Contagious organisms are well adapted to survival and growth in the mammary gland and frequently cause infections lasting weeks, months or years. The infected gland is the main source of these organisms in a dairy herd and transmission of contagious pathogens to uninfected quarters and cows occurs mainly during milking time via cloths, gloves and teat liners.

- 2) Environmental mastitis pathogens: *Streptococcus uberis*, *E.coli*, *Enterococcus* species, *Serratia marcescens*, *Arcanobacterium pyogenes*, and *Klebsiella* spp. Abbreviated hereafter as **E**.

The primary source of environmental pathogens is the surroundings in which a cow lives. Sources of environmental pathogens include manure, bedding, feedstuffs, dust, dirt, mud and water.

Low levels of some bacteria (e.g. CNS) can be treated with less urgency. However, different pathogens can have different infective doses. It is therefore important to discuss result interpretation with your vet. Presence of *Streptococcus agalactiae* should be looked at as a matter of priority. Please remember that environmental contamination could occur if poor sampling technique is used.

***It is important to discuss treatment options with your vet***

### **Guidance Notes**

**Staphylococcus aureus** - **C** - Bacteria are shed from infected quarters in low numbers. Damage to the udder tissue reduces milk yield significantly. It can **resist treatment via common antibiotics** and thus it is important to use the correct antibiotics for treatment. Culling may be an option for persistent *S. aureus* cows.

**Staphylococcus species (Coagulase Negative Staphylococci (CNS))** - **C** – Subclinical mastitis caused by intramammary infections (IMI) with coagulase-negative staphylococci (CNS) is common in dairy cows and may cause herd problems. Control of CNS mastitis is complicated by the fact that CNS contains a large number of different species and so veterinary advice should be sought. CNS is of **low pathogenicity** and is unlikely to be the sole cause of infection.

**The most likely mastitis-causing bacteria should be ruled out before CNS is considered significant in suspected mastitis cases.**

**Streptococcus agalactiae** - **C** - Very high numbers of bacteria are shed and the cell counts can be as high as 10 million without any clinical signs. Herd cell counts also increase considerably with this form of mastitis. **Careful attention to pre milking teat preparation teat dipping is essential** to minimise transmission of this organism. Treatment success rates are usually good.

**Streptococcus dysgalactiae** - **C** - is usually **associated with teat injury, either chaps or physical damage** that may be caused by **poor milking machine function**. Essential to **assess teat condition** and consider having the **parlour tested**. Infections tend to be subclinical.

**Corynebacterium bovis** - **C** - Sources of *C. bovis* are **infected udders and teat canal**. Spread of *C. bovis* is from **cow to cow at milking**. Proper **pre and post milking teat disinfection** may control the spread from cow to cow. Most cases are not clinical. **Dry cow therapy may be effective** to eliminate *C. bovis* intramammary infection.

**Streptococcus uberis** – **E/C** - A **common cause of new infection in the dry period**, and outbreaks can occur at pasture. It can produce mild to severe mastitis that can be difficult to treat. It can be shed in very high numbers by infected quarters. Sub-clinical infections may occur resulting in high cell count cows. Cow to cow transmission is also likely so good teat preparation and disinfection can be useful to minimise transmission.

**Escherichia coli (E.coli)** - **E** - Effective control includes general cow and bedding hygiene and to prevent dry cow teat end exposure. Also **pre-dipping and drying teats** prior to milking may reduce infection during lactation. *E.coli* can be found as a contaminant if samples are not taken carefully.

**Enterococcus faecalis and/or faecium** - **E** – These bacteria are **found in the gut** and are an **indicator of faecal contamination**. Recommended control procedures include **effective milking time hygiene** and pre- and post-milking teat disinfection. Also consider dry cow and cubicle bedding hygiene.

**Klebsiella spp.** - **E** – This is a member of the **coliform** family, are **found in the faeces**, in **bedding** and on **wet dirty udders**. Can cause transient, peracute or acute mastitis. They often increase in numbers in warm, wet weather.

***Serratia marcescens*** - E - Infection can be sub-clinical or clinical. Cure rates using antibiotic treatment can be variable. Transmission can be minimised by ensuring bedding and teat hygiene.

***Arcanobacterium pyogenes*** - E - Sources include wound infections, teat injuries, udder infections, abscesses, and genital tracts. Spread may be due to contact of teats with a **contaminated environment**. *A. pyogenes* may be transmitted by flies. Control measures include fly control programs, maintaining cows in clean and dry calving areas, drying up ("killing") affected quarters to reduce the risk of spreading the infection to other cows, and removing the affected cow from the herd. *A. pyogenes* often cause an acute mastitis. Infections occur most frequently in **dry cows or heifers** before calving.

#### **Penicillin Resistance: Staphylococcal beta-lactamase gene**

If a positive Staph result is detected (this applies to both *Staph aureus* and *Staph* spp. results), a beta-lactamase gene presence may be evident. This may indicate potential problems with penicillin treatment.

This is reported as follows:

<b>Result</b>	<b>Low</b>	<b>Medium</b>	<b>High</b>	<b>Interpretation</b>
NEG				<i>Susceptible to penicillin; suitable for use in treatment</i>
POS				<i>Resistant to penicillin; do not use in treatment</i>
POS				<i>Resistant to penicillin; do not use in treatment</i>
POS				<i>Resistant to penicillin; do not use in treatment</i>

Presence of the Staphylococcal beta-lactamase gene in the sample indicates there is a degree of penicillin resistance in the herd. You should discuss mastitis treatment options with your vet.

#### **Next steps**

- ***Always discuss treatment of individual cows with your vet.***
- ***If Bacillus or fungi are suspected (in rare cases), consider the use of culture testing (available through your vet).***
- ***In the case of environmental pathogens, consider reviewing milking hygiene with advice from your vet.***
- ***Monitor teat condition and discuss treatment with your vet.***
- ***Ensure milking machine function is optimal.***
- ***Results available through Micro Monitor on the Herd Companion website (via - [www.nmr.co.uk](http://www.nmr.co.uk)). Please contact customer services for your free***

## Appendix 3 – Bulk Milk Mastitis ID guidance notes

### qPCR bulk milk test result interpretation

This test is designed to detect the presence of 11 key mastitis pathogens (responsible for >95% of cases) along with penicillin resistance in *Staphylococcus* species.

Results are reported according to the level of bacteria detected in preserved milk. Bacterial growth in transit does not occur but multiple pathogens can be detected.

#### Result Interpretation – Mastitis Pathogens

Result	Low	Medium	High	Interpretation
NEG				Negative/not detected
POS	■			Positive in low numbers
POS	■	■		Positive in moderate numbers
POS	■	■	■	Positive in high numbers

Where >90% or >99% of the bacterial load is attributed to one pathogen, this will appear in the report. Pathogens can be split roughly into two groups (though some can fit into both groups):

- 1) Contagious mastitis pathogens: *Staphylococcus aureus*, other *Staphylococcal* species, *Corynebacterium bovis*, *Streptococcus agalactiae* & *Streptococcus dysgalactiae*.  
Abbreviated hereafter as **C**.

Contagious organisms are well adapted to survival and growth in the mammary gland and frequently cause infections lasting weeks, months or years. The infected gland is the main source of these organisms in a dairy herd and transmission of contagious pathogens to uninfected quarters and cows occurs mainly during milking time via cloths, gloves and teat liners.

- 2) Environmental mastitis pathogens: *Streptococcus uberis*, *E.coli*, *Enterococcus* species, *Serratia marcescens*, *Arcanobacterium pyogenes*, and *Klebsiella spp.* Abbreviated hereafter as **E**.

The primary source of environmental pathogens is the surroundings in which a cow lives. Sources of environmental pathogens include manure, bedding, feedstuffs, dust, dirt, mud and water.

Please note that environmental pathogens in the bulk tank are less likely to have come from inside the udders. Consider whether milking hygiene is satisfactory. *S.uberis* is the most likely exception to this as cow-adapted strains can be contagious.

Presence of *Streptococcus agalactiae* should be looked at as a matter of priority.

***Bulk results should not be used as a treatment guide - speak with your vet***

### **Guidance Notes**

**Staphylococcus aureus** - **C** - Bacteria are shed from infected quarters in variable numbers. Damage to the udder tissue reduces milk yield significantly. It can **resist treatment via common antibiotics**.

**Staphylococcus species (Coagulase Negative Staphylococci (CNS))** - **C** – Subclinical mastitis caused by intramammary infections (IMI) with coagulase-negative staphylococci (CNS) is common in dairy cows and may cause herd problems. Control of CNS mastitis is complicated by the fact that CNS contains a large number of different species and so veterinary advice should be sought. CNS is of **low pathogenicity** and is unlikely to be the sole cause of infection.

**The most likely mastitis-causing bacteria should be ruled out before CNS is considered significant in suspected mastitis cases.**

**Streptococcus agalactiae** - **C** - Very high numbers of bacteria are shed and the cell counts can be as high as 10 million without any clinical signs. Herd cell counts also increase considerably with this form of mastitis. **Careful attention to pre milking teat preparation teat dipping is essential** to minimise transmission of this organism. Treatment success rates are usually good but infected cows should be sought.

**Streptococcus dysgalactiae** - **C** - is usually **associated with teat injury, either chaps or physical damage** that may be caused by **poor milking machine function**. Essential to **assess teat condition** and consider having the **parlour tested**. Infections tend to be subclinical so can be monitored via bulk milk.

**Corynebacterium bovis** - **C** - Sources of *C. bovis* are **infected udders and teat canal**. Spread of *C. bovis* is from **cow to cow at milking**. Proper **pre and post milking teat disinfection** may control the spread from cow to cow. Most cases are not clinical.

**Streptococcus uberis** – **E/C** - A **common cause of new infection in the dry period**, and outbreaks can occur at pasture. It can produce mild to severe mastitis that can be difficult to treat. It can be shed in very high numbers by infected quarters. Sub-clinical infections may occur resulting in high cell count cows. Cow to cow transmission is also likely so good teat preparation and disinfection can be useful to minimise transmission. *S. uberis* can be found in bulk tank samples as a contaminant if milking hygiene processes are not maintained.

**Escherichia coli (E.coli)** - **E** - *E. coli* rarely causes persistent subclinical mastitis and thus care must be taken when interpreting bulk milk results when *E. coli* is present. It can be found in bulk tank samples as a contaminant if milking hygiene processes are not maintained. Also **pre-dipping and drying teats** prior to milking may reduce levels in the bulk tank.

**Enterococcus faecalis and/or faecium** - **E** – These bacteria are **found in the gut** and are an **indicator of faecal contamination**. Recommended control procedures include **effective milking time hygiene** and pre- and post-milking teat disinfection. Also consider dry cow and cubicle bedding hygiene.

**Klebsiella spp.** - **E** – This is a member of the **coliform** family, are **found in the faeces**, in **bedding** and on **wet dirty udders**. If found in bulk milk samples, it is likely that contamination is the cause.

***Serratia marcescens* - E** - Infection can be sub-clinical or clinical. Transmission can be minimised by ensuring bedding and teat hygiene. It is possible that this pathogen could be shed into the bulk milk from sub clinical cows.

***Arcanobacterium pyogenes* - E** - Sources include wound infections, teat injuries, udder infections, abscesses, and genital tracts. As *A. pyogenes* often causes acute mastitis, cases are often clinical resulting in milk withdrawal from the bulk tank, it is therefore unlikely to be found in bulk samples.

### **Penicillin Resistance: *Staphylococcal beta-lactamase gene***

If a positive Staph result is detected (this applies to both *Staph aureus* and *Staph* spp. results), a beta-lactamase gene presence may be evident. This may indicate potential problems with penicillin treatment for affected cows.

This is reported as follows:

<b>Result</b>	<b>Low</b>	<b>Medium</b>	<b>High</b>	<b>Interpretation</b>
NEG				Susceptible to penicillin; suitable for use in treatment
POS				Resistant to penicillin; do not use in treatment
POS				Resistant to penicillin; do not use in treatment
POS				Resistant to penicillin; do not use in treatment

Presence of the *Staphylococcal beta-lactamase gene* indicates there is likely to be some penicillin resistance in the herd. You should discuss mastitis treatment options with your vet.

***As with all results we recommend you analyse these in conjunction with your veterinarian.***

#### **Next steps**

- ***Identify 'problem' cows using NMR records and veterinary guidance.***
- ***Discuss the need for further sampling with your vet.***
- ***Send samples (kits available from NML) for qPCR testing.***
- ***Consider whether environmental pathogens are present due to poor hygiene and take relevant steps to improve this.***
- ***Results available through Micro Monitor on the Herd Companion website (via - [www.nmr.co.uk](http://www.nmr.co.uk)). Please contact customer services for your free***

## Appendix 4 – Bacto Breakdown guidance notes

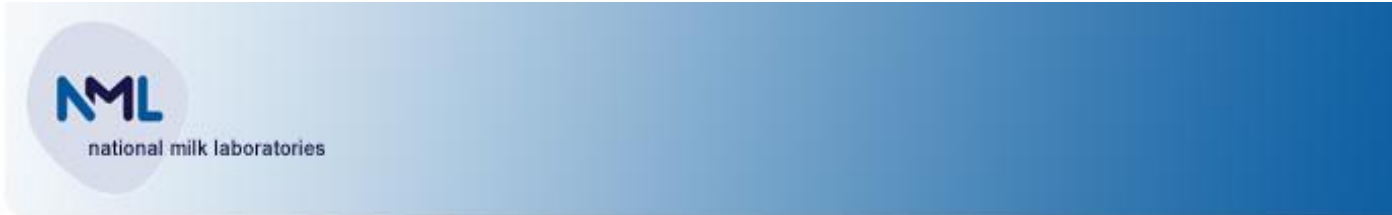
### BACTO BREAKDOWN GUIDANCE NOTES

#### PLANT HYGIENE REPORT

Test	Indicated problem	Target levels (cfu/ml unless otherwise stated)	Recommended actions
<b>Total Viable Count 30°C</b> A measure of all bacteria in the milk	Overall hygiene of production	Green <5,000 Amber 5,000-10,000 Red >10,000	Investigate bacteria from the udder/environment. check plant and/or bulk tank
<b>Bactoscan</b> (cells/ml)	Overall hygiene of production	Green <20,000 Amber 20,000 - 50,000 Red >50,000	Investigate bacteria from the udder/environment. check plant and/or bulk tank
<b>Somatic Cell Count</b> (cells/ml)	Sub-clinical mastitis	Green <150,000 Amber 150,000 - 250,000 Red >250,000	Review of mastitis management to prevent spread of infection
<b>Coliforms 30°C</b> Environmental contamination	Poor teat preparation	Green <20 Amber 20-40 Red >40	Check plant cleaning. Review pre-milking teat preparation (Pre-dipping recommended)
<b>Thermotolerants</b> Bacteria that withstand high temperatures and grow in dirty milking systems	Inadequate plant cleaning	Green <150 Amber 150 - 500 Red >500	Poor plant cleaning. Ensure the boiler is heating correctly, check wash concentrations, volumes of solution etc.
<b>Psychrotrophs 22°C</b> Bacteria able to grow at refrigeration temperatures (can be found in water and bedding)	Inadequately cleaned bulk tank, poor milk cooling and/or dirty bedding	Green <500 Amber 500-5,000 Red >5,000	Check milk cooling and bulk tank cleaning. Review pre-milking teat preparation

FURTHER ADVICE ON INTERPRETING THESE RESULTS PLEASE CONSULT YOUR VET OR MILK QUALITY CONSULTANT

For more information on any of our services please call customer services on 0844 725567



[www.nationalmilklaboratories.co.uk](http://www.nationalmilklaboratories.co.uk)