

Human infection and ethanol plant contamination

An analogous visit to the doctor's office
with questions and expectations

2007 International Fuel Ethanol Workshop & Expo
St. Louis, Missouri, USA

June 27, 2007

Dr. Dennis Bayrock

Seminar outline

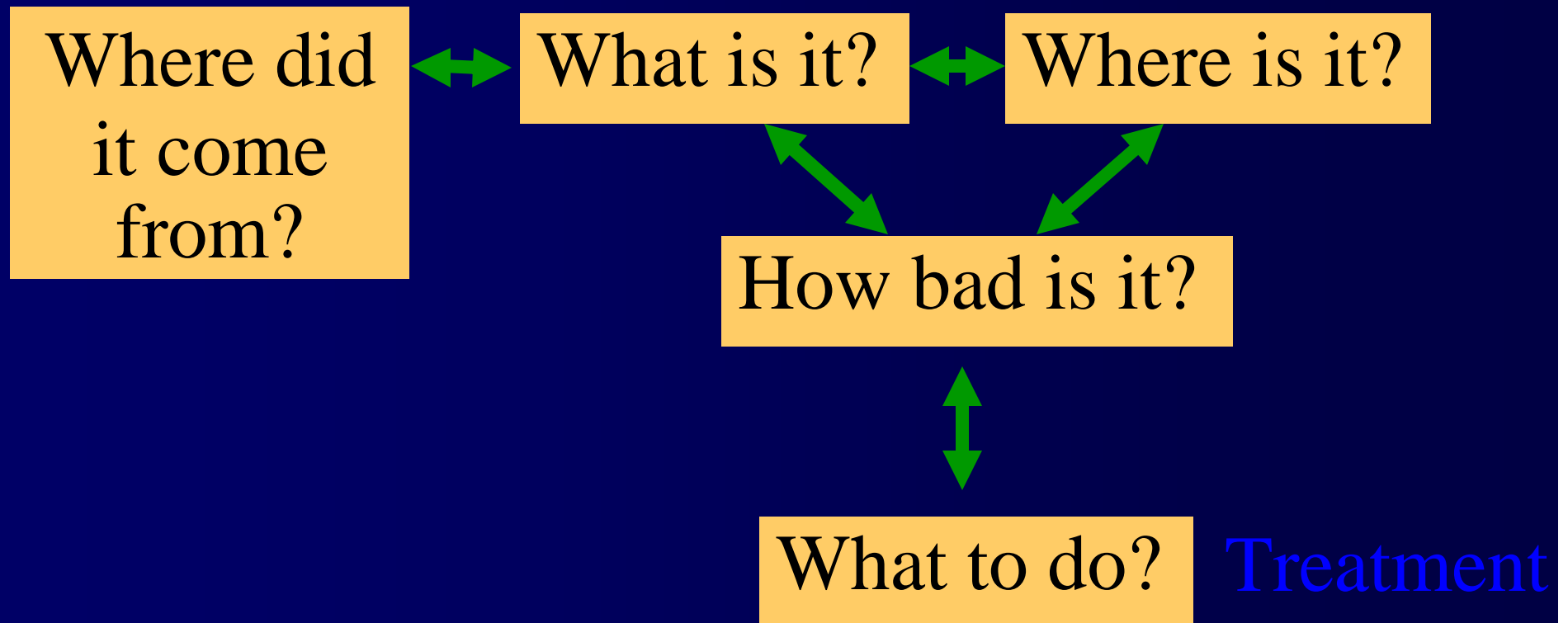
- Analogies between Human health and Ethanol plant health
- What is it?, Where is it?, How bad is it?
- Visit to the Doctor's office
 - Patient reaction and questions asked.
 - Diagnosis
- Why viable counts?

Seminar focuses on concepts we use in our own health and how it should be applied to industry

Expectations

Source

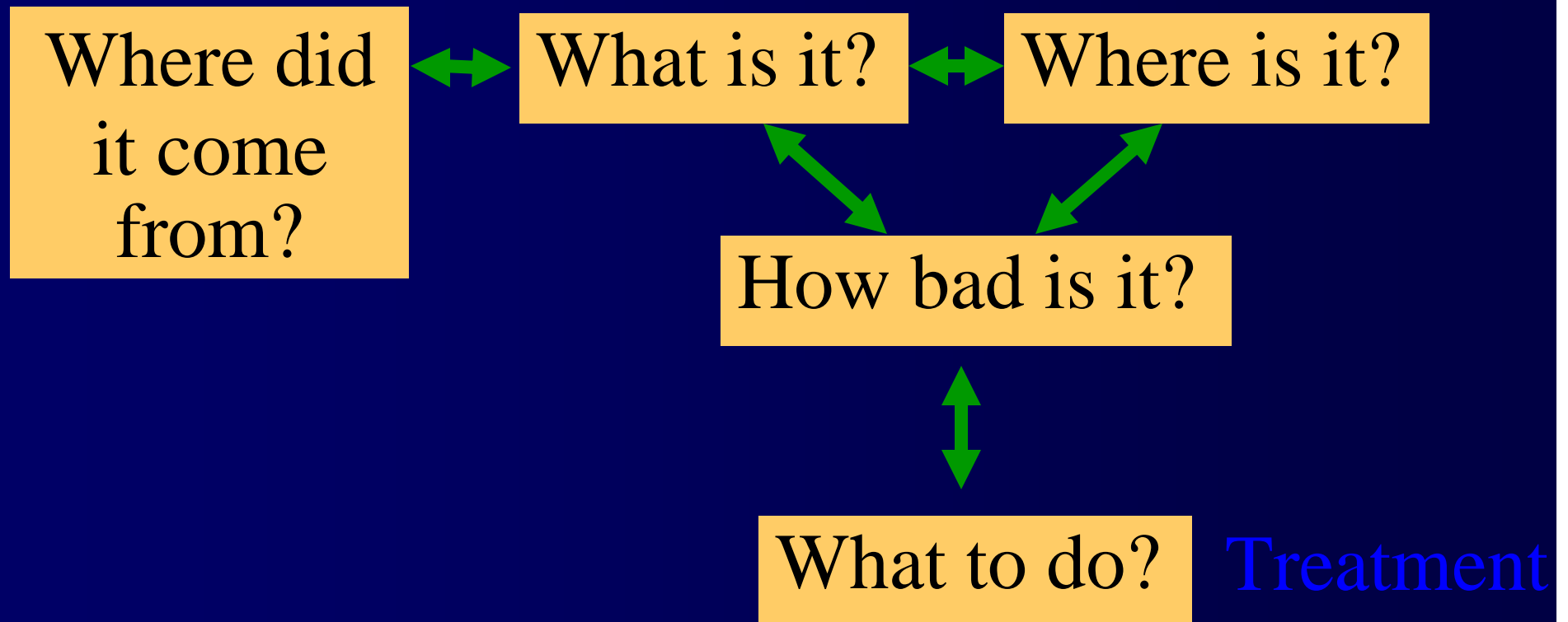
Diagnosis



Expectations

Source

Diagnosis



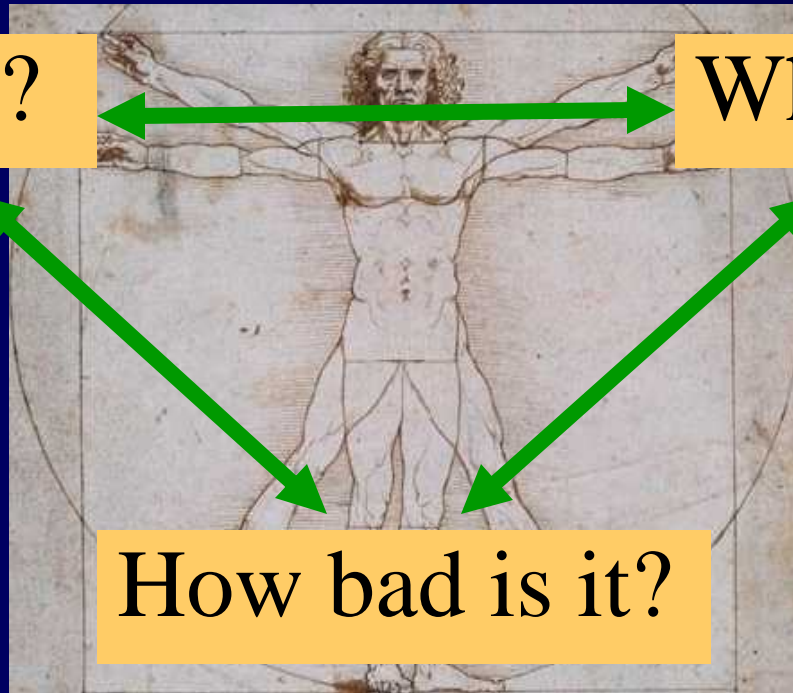
Diagnosing contamination

To understand human infection, your doctor needs to know:

What is it?

Where is it?

How bad is it?



Diagnosing contamination

To understand human infection, your doctor needs to know:

e.g. AIDS, *Mycobacterium tuberculosis*
Streptococcus pneumoniae

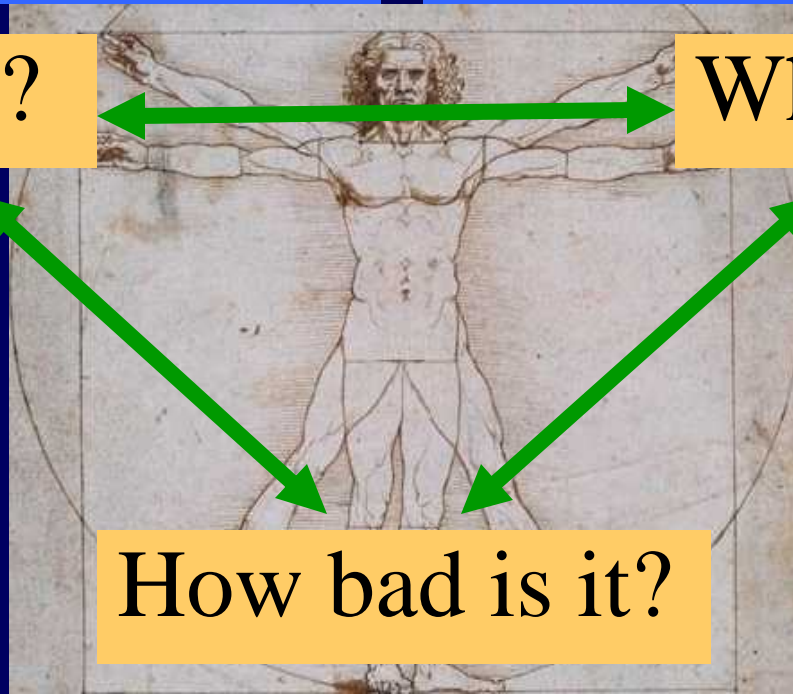
e.g. Localized in your arm? In your blood?
In your nasal passages?

What is it?

Where is it?

How bad is it?

Small infection?, Raging infection?, Scratch?
Septicaemia?, Life threatening?

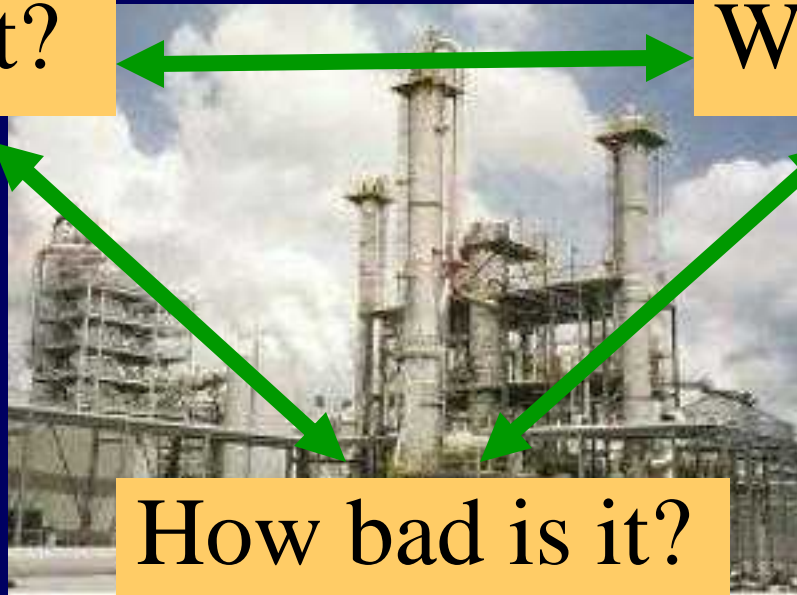


Diagnosing contamination

To understand infection at an ethanol plant, one needs to know

What is it?

Where is it?



How bad is it?

Diagnosing contamination

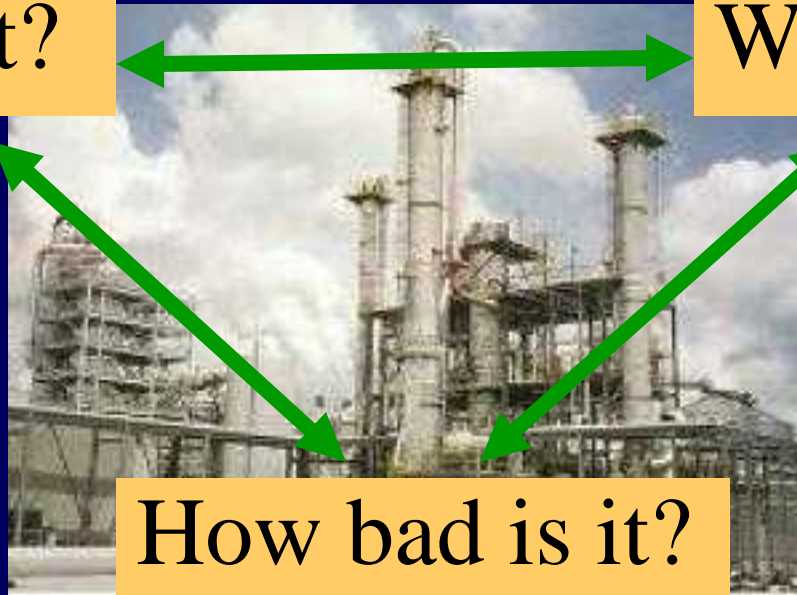
To understand infection at an ethanol plant, one needs to know

e.g. *Lactobacillus plantarum*
Lactobacillus paracasei

e.g. Located in your Propagators? Fermentors?
Recycle water tank?

What is it?

Where is it?



How bad is it?

Are the numbers “below the radar” for influencing yeast? Ethanol yields go down?

Diagnosing contamination



What is it?

Lactic Acid Bacteria (LAB) family *

Lactobacillus sp

paracasei, plantarum, casei, brevis, fermentum, rhamnosus, delbrueckii, buchneri, pentosus, acidophilus, gasseri, jenserii, amylovorus, reuteri, cornyeformis, divergens, carnis, piscicola, sake, sharpeae, bavaricus, curvatus, hamsteri, amylophilus, agilis, homohiochii

Weisellia sp

paramesenteroides, confusa, viridescens

Lactococcus sp

lactis, raffinolactus, plantarium

* Approx 70% of infections at an ethanol plant are due to LAB (Skinner and Leathers, 2003; Bayrock, unpublished results)␣

Diagnosing contamination



What is it?

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paramesenteroides, confusa, viridescens

Lactococcus sp

lactis, raffinolactus, plantarium

Not all of these bacteria are present at the same time!

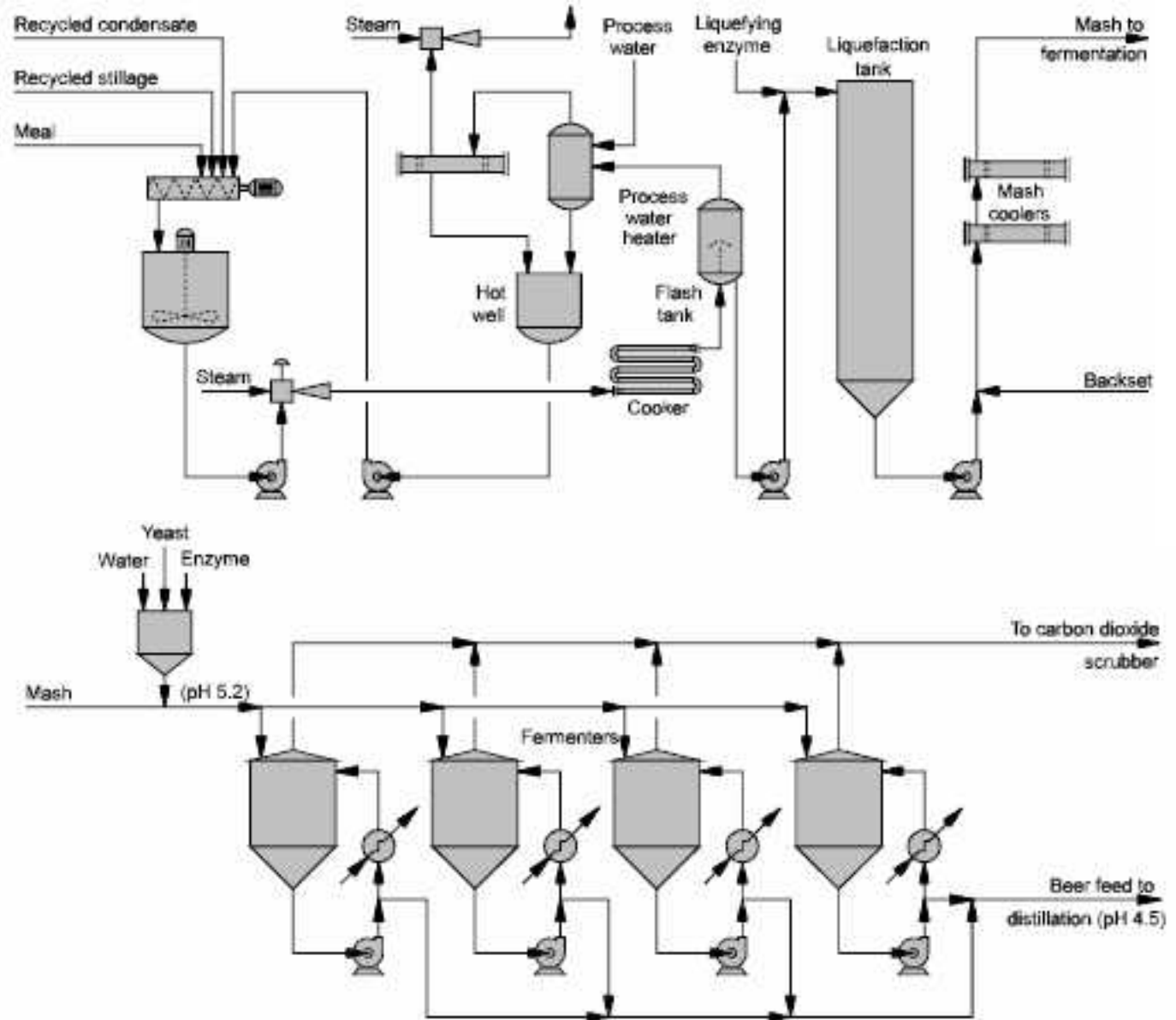
If a microbiologist processes enough mash and has enough time, these bacteria (and probably others) can be found.

Question: If you find the presence of *L. lactis* at a plant, is it relevant?
i.e. “ I found <insert microbe ID here> in your plant”

* Approx 70% of infections at an ethanol plant are due to LAB (Skinner and Leathers, 2003; Bayrock, unpublished results)␣

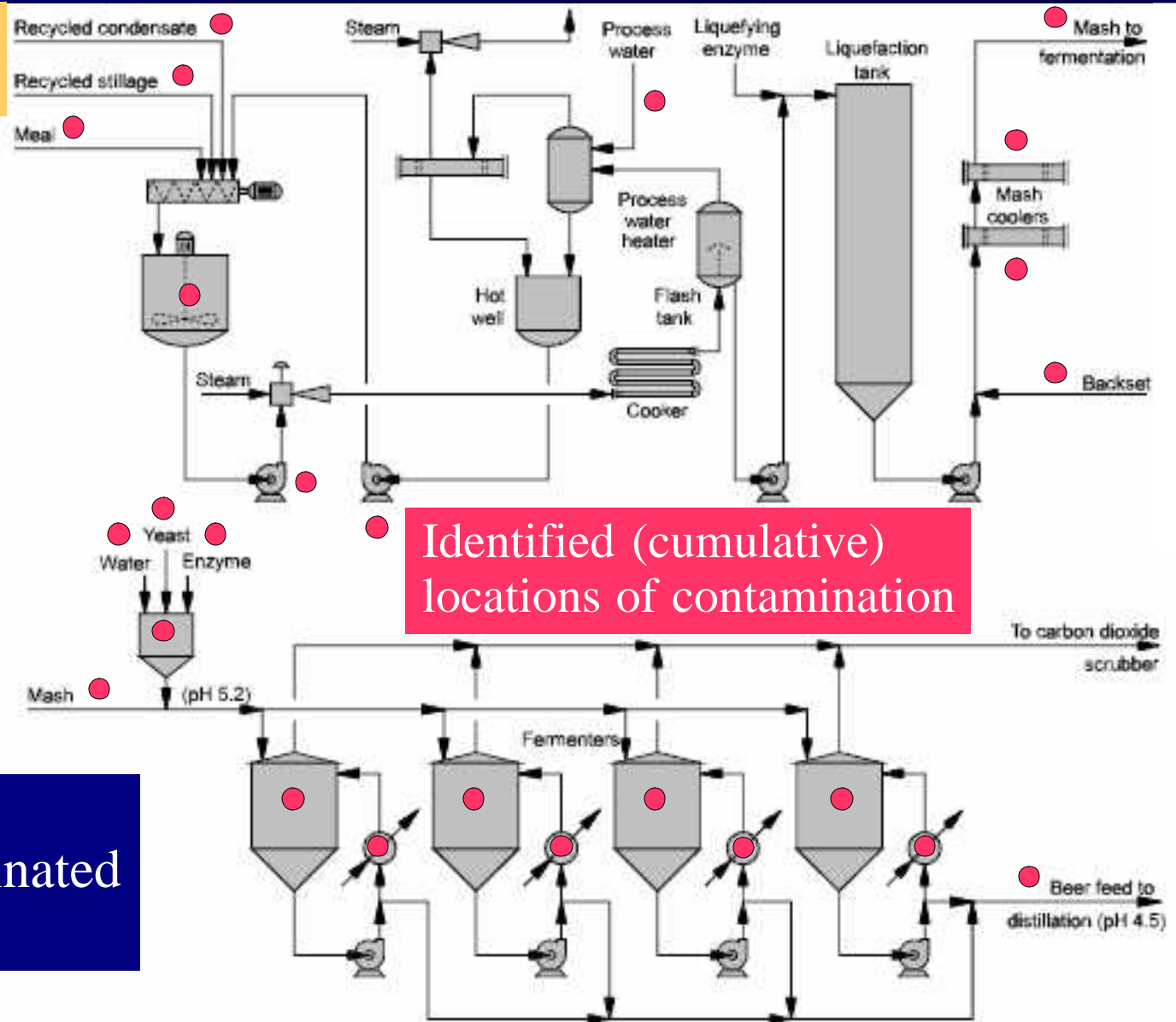
Understanding contamination

Where is it?



Understanding contamination

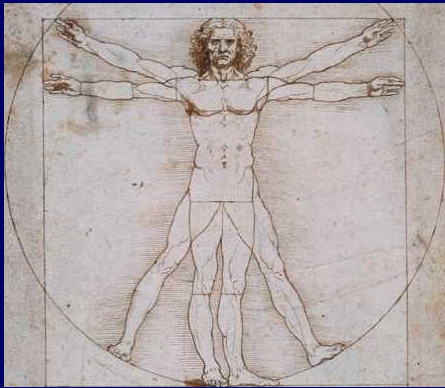
Where is it?



Not all of these locations contaminated at the same time!

Understanding contamination

How bad is it?



For Humans:

- In general, the severity of pain, disorientation, etc the patient is experiencing can provide some answers.
- Additional tests are usually done.



For an Ethanol Plant:

- Traditional: HPLC (ethanol loss, lactic/acetic acids), microscope counts
- Best indication of contamination?

Viable counts

Understanding contamination

How bad is it?



An analysis of over 1000 samples from contaminated ethanol plants (together with plant process data) confirms that in most cases:

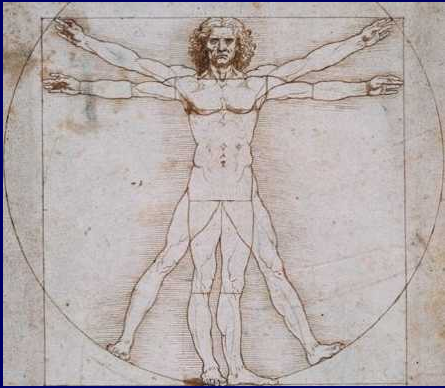
1×10^5 viable bacterial cells/ml mash (CFU/ml)

Is a critical (threshold) contaminant viability level!

Levels of viable bacteria above this level will likely begin to:

1. Decrease final ethanol yield.
2. Decrease yeast viability and vitality.
3. Slow down rate of fermentation
4. Increase lactic/acetic acids.

Tying together “What” “Where” and “How bad is it”

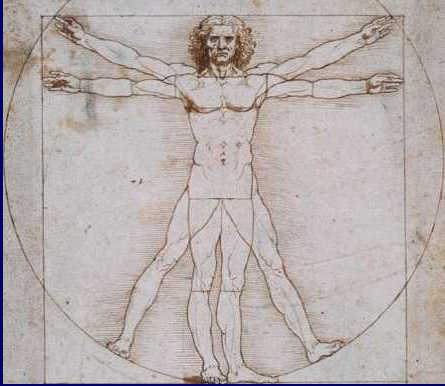


A visit to the doctors office

Situation:

A person pays a visit to their local clinic.
The doctor runs some tests and is
consulting with the patient

Tying together “What” “Where” and “How bad is it”



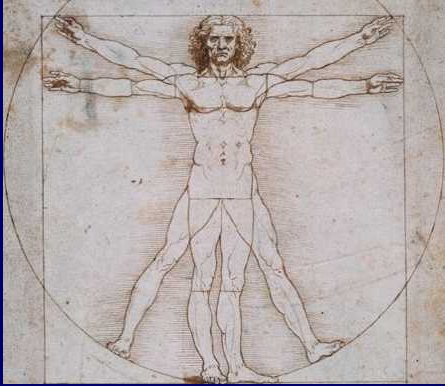
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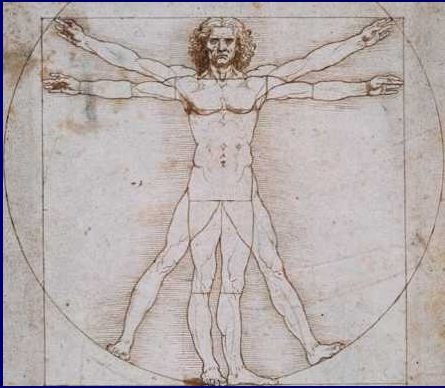
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Doctor: “Your tests have come back - I'm sorry to say you have an infection”

- High stress for patient.
- What would be your first reaction? - Fear? Shock?
- Would you expect the doctor to stop here?
- What would be your first question to the doctor?

Tying together “What” “Where” and “How bad is it”



A visit to the doctors office

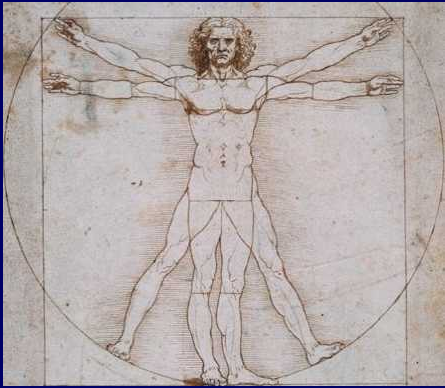
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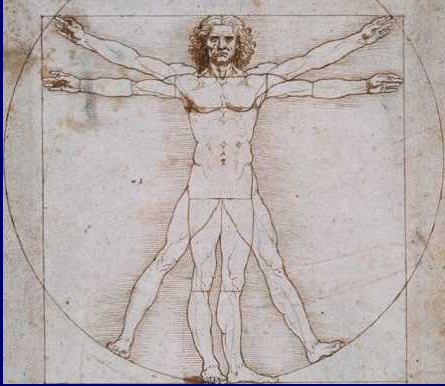
Doctor: “Your tests have come back - I'm sorry to say you have an infection”

Patient: “Where is it?”

Doctor: “It's an infection on your skin”

- Would it make a difference if it was in the blood?
- A more serious infection?

Tying together “What” “Where” and “How bad is it”



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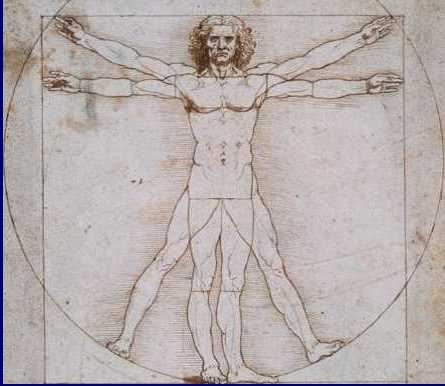
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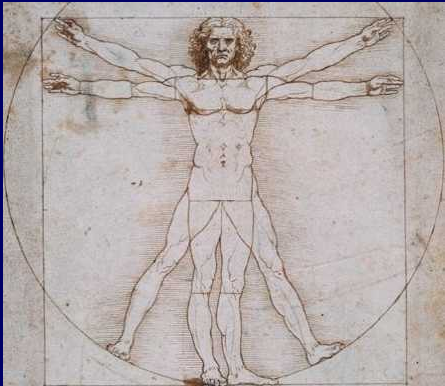
Doctor: “It's an infection on your skin”

Patient: “How bad is it?”

Doctor: “I can treat your acne with antibiotics”

- Lower stress for patient.
- Not a serious life-threatening situation

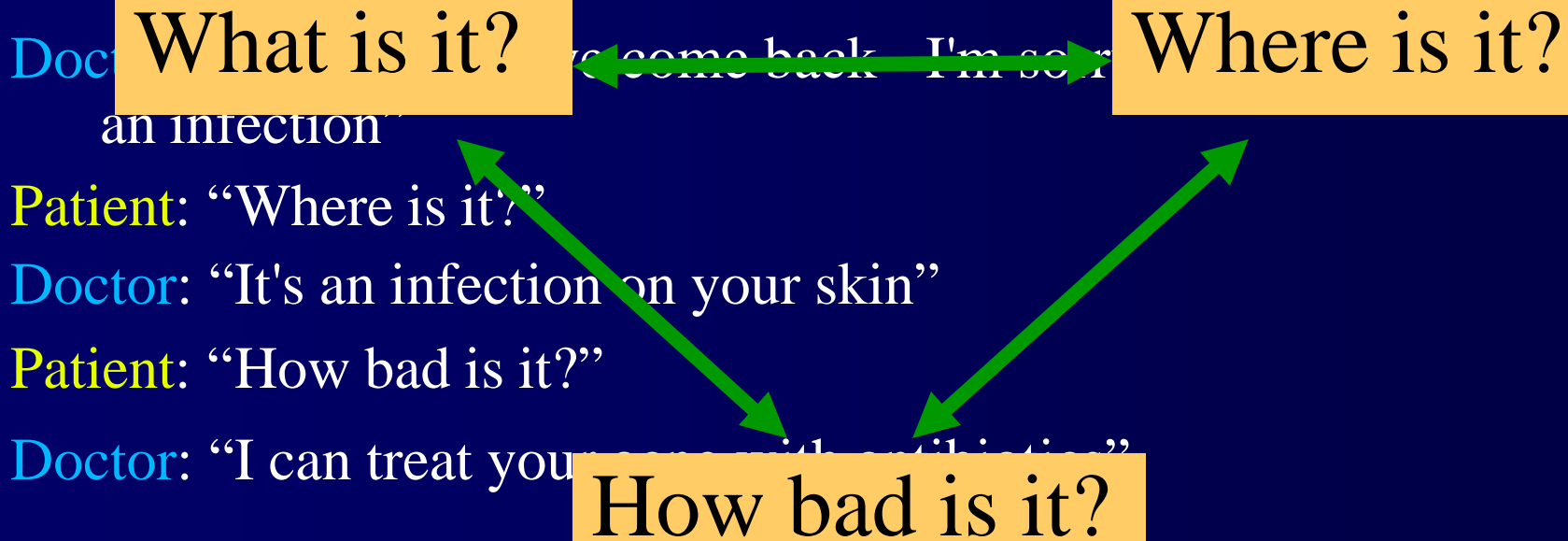
Tying together “What” “Where” and “How bad is it”



A visit to the doctors office

Situation:

A person pays a visit to their local clinic.
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Diagnosis is complete

Tying together “What” “Where” and “How bad is it”



An infected ethanol plant

Situation:

A PhibroChem representative debriefs a client at a contaminated ethanol plant.

PhibroChem: “You have a *Lactobacillus* sp infection”

Tying together “What” “Where” and “How bad is it”



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Tying together “What” “Where” and “How bad is it”



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Client: “Where is it?”

PhibroChem: “It's in your Propagator”

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PhibroChem: “You have a *Lactobacillus* sp infection”

Client: “Where is it?”

PhibroChem: “It's in your Propagator”

Client: “ How bad is it?”

PhibroChem: “ The infection level (live bacteria) is at 1×10^4 CFU/ml”

- Lower stress for client.
- Not a serious “plant-threatening” situation

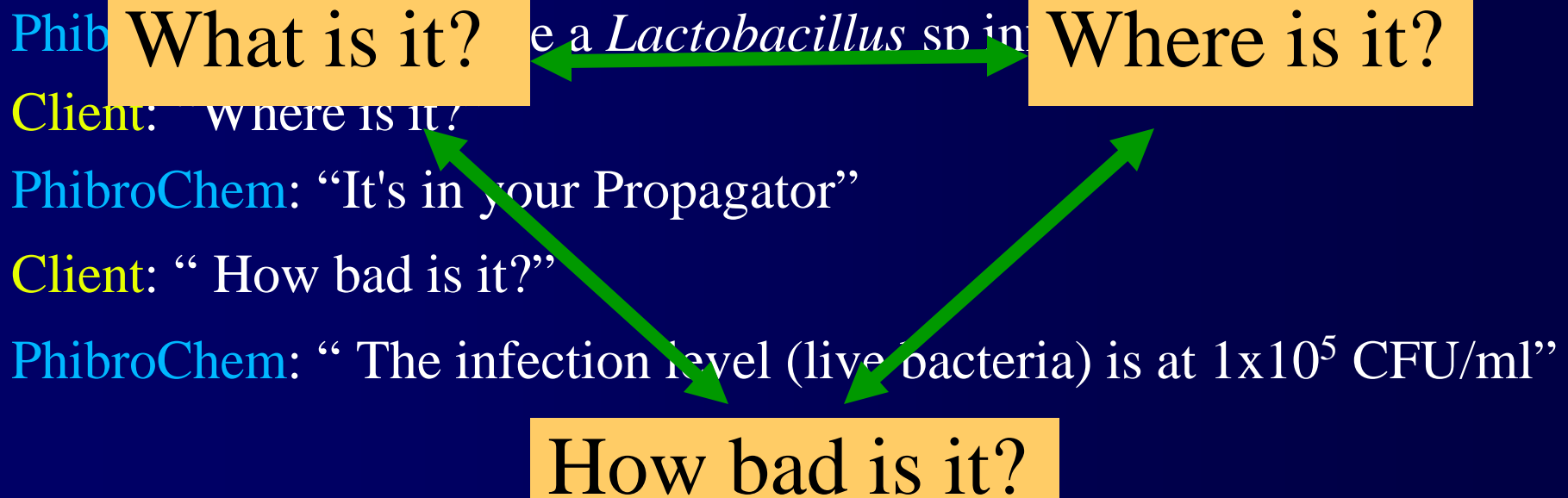
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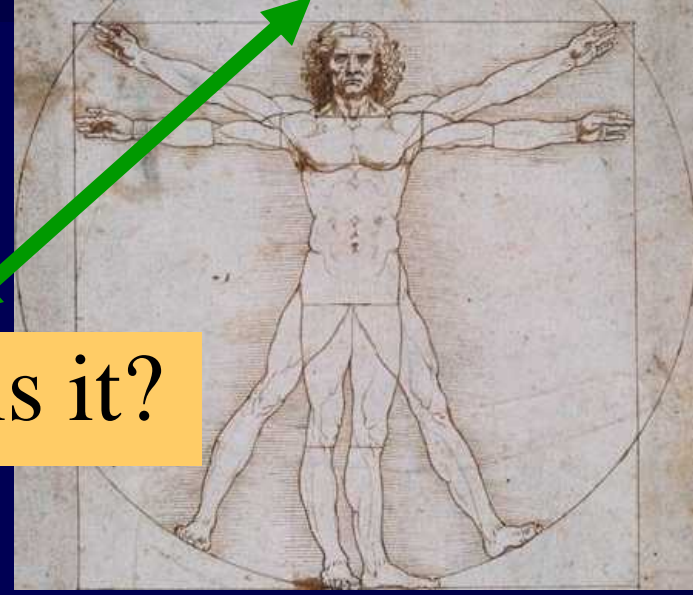


Diagnosis is complete

Tying together “What” “Where” and “How bad is it”

What is it?

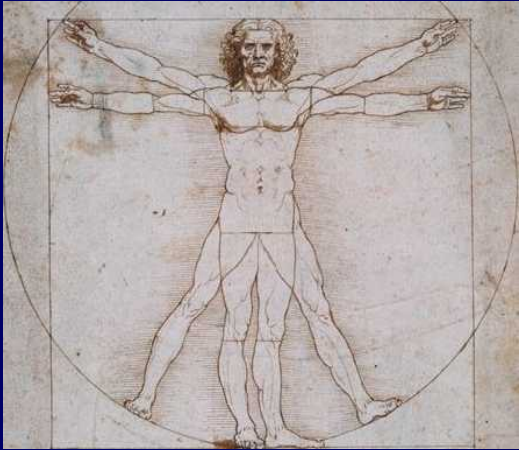
Where is it?



How bad is it?

Diagnosis can indicate Life-threatening (“Plant-threatening”)
situation or a less serious problem (as posed by above examples)

Specific example of “What” “Where” and “How bad”



[Clinic Standard: HYPERLIPIDEMIA SCN]

Patient Id: 082136
First Name: DENNIS P
Last Name : BAYROCK
Address :

Default Provider: 15 - C.S. BUTTINGER
Referring Doctor:

Collection Dates	2003 Oct 16	2005 Feb 04
Triglycerides	H	
mmol/L 0.60-2.30	3.27	1.86
Cholesterol HDL		
mmol/L 0.90-1.60	0.94	0.97
LDL Cholesterol	H	H
mmol/L 2.00-3.40	4.15	4.91
Cholesterol Total	H	H
mmol/L 3.80-5.20	6.58	6.73
Risk Ratio		
	7.0	6.9

Specific example of “What” “Where” and “How bad”



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Cholesterol Total
mmol/L 3.80-5.20
Risk Ratio

H
3.27 1.86
0.94 0.97
H **H**
4.15 4.91
H **H**
6.58 6.73
7.0 6.9

Where is it?

(Blood)_p

How bad is it?

What is it?

Specific example of “What” “Where” and “How bad”

Sample PhibroChem contamination report



Microbe Type		Sample					
		5233	5234	5235	5236	5237	5238
Yeasts	Total (production)	-	2.70E+07	2.10E+08	-	1.56E+05	1.40E+04
	Wild (Lysine)	-	-	-	-	-	-
	Wild (LWYM)	-	-	-	-	-	-
	<i>Brettanomyces/Dekkera</i>	-	-	-	-	-	-
Bacteria	Total aerobic	-	4.60E+05	1.00E+05	1.20E+04	1.00E+05	2.00E+04
	Total anaerobic	-	5.20E+06	1.10E+07	2.00E+04	1.20E+05	4.70E+05
	Lactic acid bacteria	-	4.80E+06	5.60E+06	2.20E+04	1.30E+04	5.70E+05
	Gram -ve	-	-	-	-	-	-

All numbers expressed as viable CFU/ml

Legend: - Not detected above 10^2 CFU/ml
 † High level of microbe - Increased risk to fermentation

PhibroChem's diagnostic lab

- Operating for 6 years
- In 10 month operation:
 - 1200 Client samples processed
 - 40,000 Microbiological plates processed
- Estimate for 6 year operation:
 - 200,000 Microbiological plates processed

Specific example of “What” “Where” and “How bad”

Where is it?

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	Wild (Lysine)	-	-	-	-	-	-
	Wild (LWYM)	-	-	-	-	-	-
	<i>Brettanomyces/Dekkera</i>	-	-	-	-	-	-
Bacteria	Total aerobic	-	4.60E+05	1.00E+05	1.20E+04	1.00E+05	2.00E+04
	Total anaerobic	-	5.20E+06	1.10E+07	2.00E+04	1.20E+05	4.70E+05
	Lactic acid bacteria	-	4.80E+06	5.60E+06	2.20E+04	1.30E+04	5.70E+05
	Gram -ve	-	-	-	-	-	-

All numbers expressed as viable CFU/ml

Legend: - Not detected above 10^2 CFU/ml
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What is it?

How bad is it?

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Viability

Why has PhibroChem standardized their diagnostic kits on microbiological plate counts?



Developed initially by Robert Koch (1843-1910)‡

Widely considered one of two
“Founding Fathers” of Bacteriology

(the other being Louis Pasteur)‡

- Since Koch, plate media have been refined, researched, formulated, (and commercialized) to detect and differentiate THOUSANDS of bacteria. Over 3000 different types of media have been formulated and can be ordered.

Viability

Why has PhibroChem standardized their diagnostic kits on microbiological plate counts?

- Plate counts are the “Gold Standard” method for determining viable cells. All other developed methods are compared to plate counts.
- In Hospitals, plate counts are the primary method to diagnose any type of infection.
- Easy to perform (after appropriate training)
- Provides reliable and consistent viable counts (dead cells not counted)
- Mash particles do not interfere with viable counts
- Methodology well understood
- Can determine identity of bacteria.
- Low capital investment.

Viability

Why has PhibroChem standardized their diagnostic kits on microbiological plate counts?

Primary disadvantages:

- Time consuming to prepare materials and to perform test
- Results cannot be provided in “real time”
- Plating must be done within a few days of sampling

No significant difference in viable counts (90% confidence level) if samples are plated within 3 days of sampling (with proper sample management)

Tying together “What” “Where” and “How bad is it”

Conclusions:

1. It is not good enough to know “What is it?” and “Where is it?” as this does not give a complete diagnosis of health.
2. How one reacts and questions their medical doctor is analogous to situations at an infected ethanol plant.
3. Too much emphasis has been placed on “What is it?” and “Where is it?” and not enough on “How bad is it?”
4. Finding “X” contaminant at an ethanol plant does not necessarily make it relevant

Tying together “What” “Where” and “How bad is it”

Conclusions:

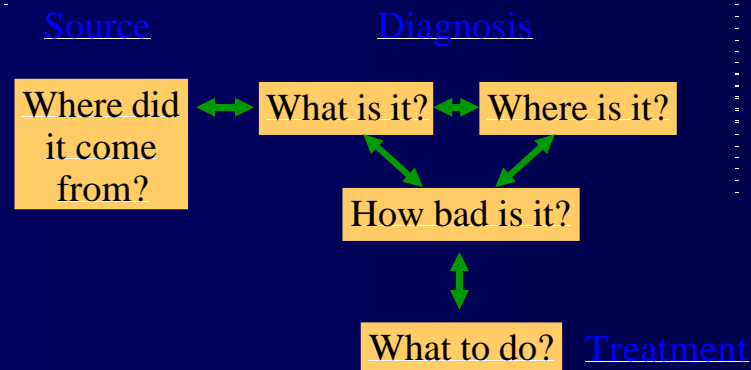
5. Live bacteria (not total) are what influences yeast and impacts an ethanol plant's productivity.
6. Indication of infection can be seen by HPLC (lactic/acetic acids). Severity of infection best assessed by **live counts**.

Different species of *Lactobacillus* produce different levels of lactic/acetic acids.

1×10^6 CFU/ml <i>Lactobacillus fermentum</i>	---> 0.3% w/v lactic acid
1×10^6 CFU/ml <i>Lactobacillus plantarum</i>	---> 0.1% w/v lactic acid

For the health of your plant:

“Step back and examine the whole picture”



Thank you for your time!