Microbiology for Non - Microbiologists

AN INTRODUCTION TO MICROBIOLOGICAL CONCEPTS AND WAYS TO REDUCE CONTAMINATION

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Introduction

- Welcome
- Who is your presenter?
- What's in it for you?
- Why is this training needed?

Learning Aims

The aim of this training is to provide an introduction microbiology by examining:

- What is Microbiology
- Why contamination is undesirable
- Where microbes are found in nature
- Ways to reduce or control microbe numbers:
  - Sanitisation
  - Sterilisation
  - Reduce particles
  - Keeping objects dry
  - Hindering growth
  - Aseptic practices

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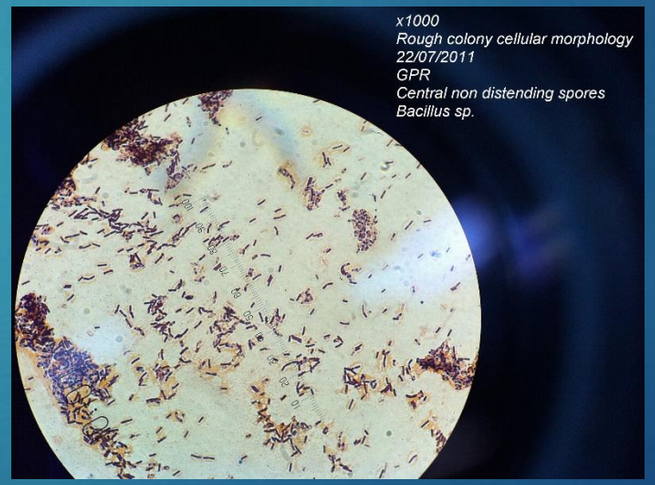
Learning Outcomes

At the end of this session the you will be able to identify:

- The differences between sterile and sanitised
- Methods used to control the numbers of microbes
- Where one may find microbes
- The largest source of clean room contamination
- Why keeping objects dry is important in controlling microbes
- What makes a microbe a ‘bad’ microbe

What Is Microbiology?

- Microbiology is the study of living things that are too small to see. These are called microbes.
- In order to see microbes we use a microscope. This magnifies up to 1000X.



Why Contamination Is Undesirable

As of March 4 2020, 16 of the last 50 recalls by the FDA for pharmaceutical products were due to microbial contamination or a lack of sterility assurance. Another 27 were due to impurities with 5 more due to mislabelling.

Looking at TGA recalls for 2019, around half of the medicine recalls due to contamination were the result of microbial contamination.

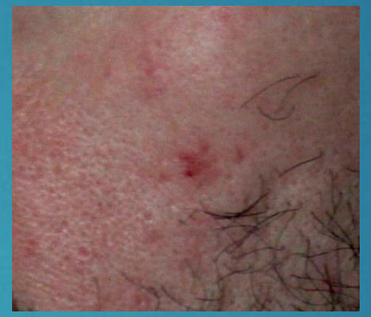
- The contamination consisted of either macro (glass, metal, plastic fragments) or micro (microbial and allergenic (aka chemical)) matter.

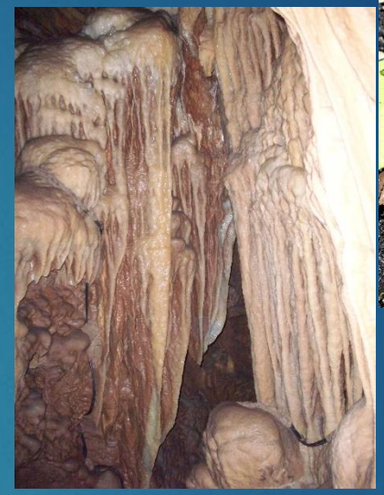
Contamination results in a substandard product that may or may not result in undesirable (or unintended) outcomes.

- Businesswise, contamination equals:
  - A poor product
  - Recalls
  - Customer loyalty diminishes
  - A bad recall can cripple a company

Where Are Microbes Found In Nature?

- Everywhere there is free water, living microbes have been discovered:
  - Deep see vents, caves, hot springs
  - Sinks, wet surfaces, drains
  - Soil
  - Skin
  - Plants
  - Air
- Microbes can be very hardy
- Microbes are found practically everywhere





Microbe Housing

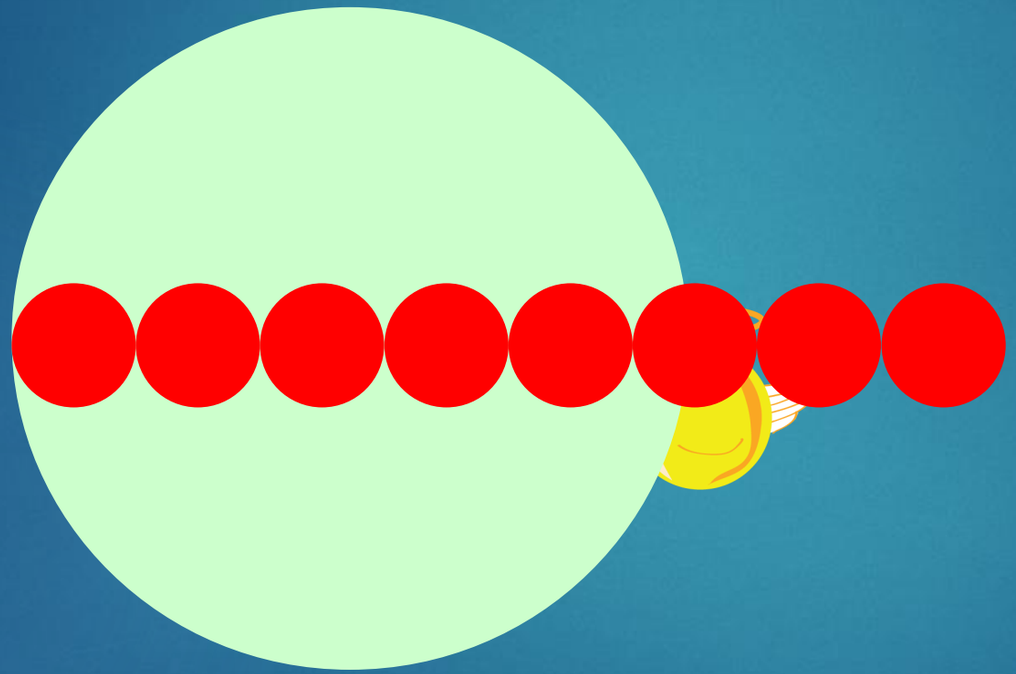
- Large intestine:
  - 1x 10^{11} microbes per gram (wet weight) for a total of 1x 10^{14} -

    100 000 000 000 (wet) - 100 000 000 000 000 (dry)
- Surface of skin:
  - area of approximately two square meters per adult:
  - Approx 1x 10^{14} bacteria spread over it (concentrated in oily regions and sweaty regions) - 100 000 000 000 000
- Oral cavity:
  - 1x 10^{10} per ml of saliva - 10 000 000 000
- The human body is comprised of approximately 1x 10^{13} to 1x 10^{14} cells - 10 000 000 000 000 to 100 000 000 000 000

- This is why it is advisable to wash your hands before eating!

Size Of Microbes

- Due to their size, microbes are only visible in large numbers or when they are concentrated in a small area



- "Good" vs "Bad" microbes

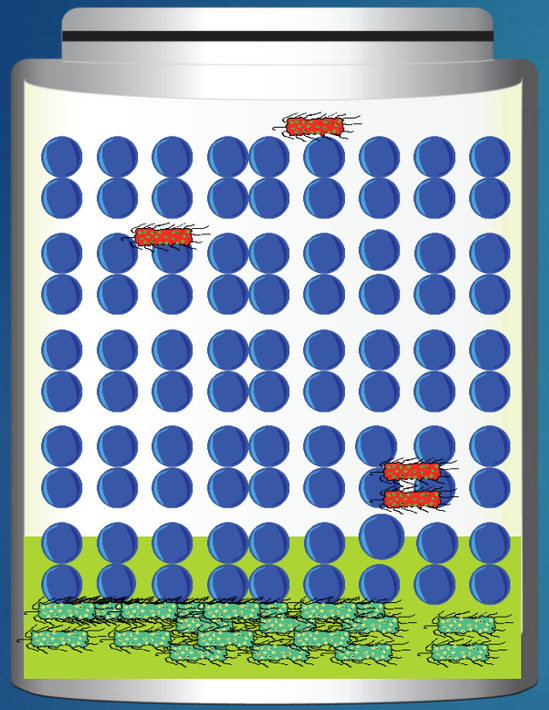
When Microbes Go Bad

- How Microbes go bad.
  - Wrong place
  - Too many
  - Wrong type
- Why would microbes in product not be a good thing?
  - Reduce the effectiveness of product
  - Sicken or kill patient

What Might A Microbiology Lab Test?

- Raw materials and packaging
- Bulk product samples
- Packed product samples
- Room air & surfaces
- Equipment
- Water for Injection or Purified Water
- Endotoxin presence or absence
- Limits:
  - <100cfu/10g or item
  - Absence of certain microbes
    - Pseudomonas, Escherichia Coli, Salmonella, Staphylococcus aureus, Candida
- Testing does not eliminate or control microbes.
- Limitations of testing

Microbes In Product



Bulk Mixing Vessel

Product Containers

Micro Sample

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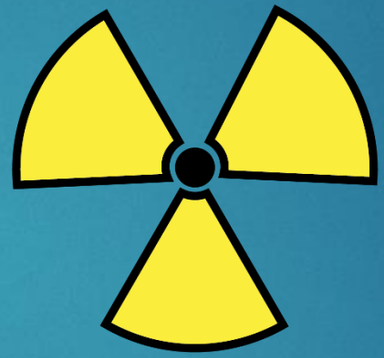
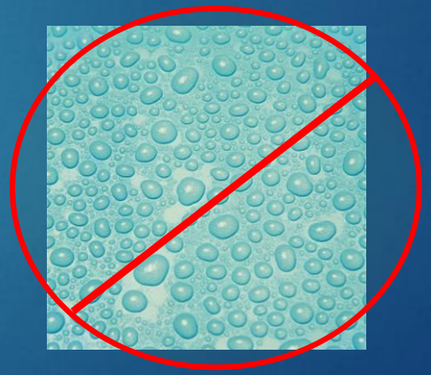
Control Of The Environment & Manufacturing Process

- Everything is planned and follows written instructions
- Special rooms used to manufacture
- Personnel movements are controlled
- Raw materials and finished product is examined
- Microbes are reduced or controlled through various processes

Ways To Reduce Or Control Microbe Numbers

- Microbes numbers can be reduced or controlled by:
  - Sterilisation
  - Sanitisation
  - Reducing particle generation
  - Hindering the growth potential of microbes
  - All of the above - Aseptic Practices





Aseptic Practices

- Used to prevent or reduce the potential for contamination
  - Excludes, removes or kills microbes
    - Eg sampling a product aseptically will mean the sample tested by micro will represent the batch
  - Is the combined result of sterilisation, sanitation, particle reduction and cleaning
    - Eg: aseptic practices in a kitchen lead to will result in less chance of unexpected surprises or tastes in your food. In your plant they will lead to quality products and less microbes.

Sanitisation vs Sterilisation

- Sanitisation:
  - Reduces the number of living organisms
  - Eg: washing hands, cleaning floors, alcohol & disinfectant (Marinol / Process / Hypochlorite)
- Sterilisation:
  - totally eliminates microbes
  - Eg: pressure cooker (Autoclave), γ (gamma) irradiation, sterile filtration, gases (ETOH, Formaldehyde)

Reducing Particles

- Where are microbes found?
  - On people
  - In the air
  - On surfaces
- Particles may be microbes (or have microbes attached)
- How to reduce the impact of particles?
  - Filters
  - Fabrics
  - Containment
  - Jewellery and cosmetics
  - Maintain health

Hindering Growth

- Microbes need water and food to grow:
  - Remove water
  - Remove food
- Chemicals can also restrict growth
- Cleaning/Sanitation – clean, rinse & **DRY**

Sterile Manufacture *Environmental Monitoring*

- The following pages are specific to plants where drugs are made in a sterile facility.
  - Topics
    - Media Fill Trials
    - Gowning
    - WFI
    - Endotoxin Testing
    - Sterility Test

Media Fill Trials

- For sterile manufacturers, an important process check is the media fill trial.
  - Uses microbiological media in place of product
  - Simulates filling process
  - Vials incubated and 100% visually examined
  - Requirements for batches where <5000 units are filled differ to 5000+ with regards to allow positive vials
  - Here effect on integrity of batch of deviations from non documented procedure can be investigated

Aseptic Gowning

- Purpose
- Particle containment
- Airlock System / step overs
- Behaviour when in clean room:
  - Minimise movement and talking
  - Where can go in clean room
  - Movements restrictions while manufacturing
  - etc

Environmental Monitoring

- Water For Injection (WFI)
- Clean Rooms
  - Room classifications:
    - Class 100, 10 000, 100 000
    - Class A, B, C, D
  - Air gradients
  - Types of monitoring and frequency

Endotoxin Testing

- What are endotoxins
- Why are they biologically significant
  - WFI testing
  - Product Testing
- How to remove them?
- Consequences if injected into patient

Sterility Testing

- Aseptically filled products are tested for complete absence of microbes
  - Must be 100% free of microbes
  - Does not ensure quality of product, this needs be built into the whole manufacturing process
  - A failure could indicate serious problems in manufacturing processes

Sterile Summary

- Differences between aseptic vs non aseptic
- More EM testing
- Product testing differences
- Endotoxins
- Testing is a process check and is not a control

Time to Experiment

Aim:

- to recover microbes from your skin and person.

Materials:

- Contact plates
- Petri dishes
- Swab
- Hand sanitiser
- Boot cover

Results:

- A follow-up session will be held next week to view and discuss the results of today's testing

Experiment Follow-up

- Samples taken today will be incubated
- Where results can be seen
- What should the results show us?

Knowledge Check Q1

(non sterile section)

- What is Microbiology?
- a/ the study of small goldfish
- b/ the study of living things too small to see with the naked eye
- c/ putting small computer chips inside people
- d/ all of the above

Knowledge Check Q2

(non sterile section)

- How can you see a single microbe?
- a/ organise to go on a date with it
- b/ put it under a magnifying glass
- c/ put it under a microscope at high magnification
- d/ b and c

Knowledge Check Q3

(non sterile section)

- How big are bacteria?
- a/ as big as a SUV
- b/ as big as an eyelash
- c/ 8x smaller than the width of a human hair
- d/ 5x smaller then the width of an animal cell

Knowledge Check Q4

(non sterile section)

- What is the difference between sterile and sanitised?
- a/ sterile is devoid of life, where sanitised is a G rated movie
- b/ sterile = no living things, sanitised = a reduction in the normal number of living things
- c/ the opposite of answer b
- d/ they are both the same

Knowledge Check Q5

(non sterile section)

- Where can you find microbes?
- a/ under your fingernails
- b/ at the bottom of the ocean
- c/ floating in the air
- d/ on your clothes
- e/ all of the above

Knowledge Check Q6

(non sterile section)

- What is the largest source of microbes in a cleanroom?
- a/ burrowing animals
- b/ insects
- c/ humans
- d/ generic pharma spies

Knowledge Check Q7

(non sterile section)

- What is the best way to personally control spreading microbes?
- a/ wrap yourself in plastic
- b/ use tongs to touch everything
- c/ don’t breath on your lunch
- d/ wash your hands

Knowledge Check Q8

(non sterile section)

- What do bacteria need to multiply?
- a/ a bacterium of the opposite sex
- b/ a how to book
- c/ food, water and a suitable temperature
- d/ a calculator with tiny bacteria sized buttons

Knowledge Check Q9

(non sterile section)

- What are aseptic practices?
- a/ Using sterilisation
- b/ Using sanitation
- c/ Providing a barrier between you and the product being manufactured
- d/ Reducing the amount of food and water available to microbes
- e/ Practicing building a septic tank

Knowledge Check Q10

(non sterile section)

- How do we control microbe numbers?
- a/ use aseptic practices
- b/ willing the microbes not to multiply
- c/ use documented cleaning and manufacturing procedures
- d/ all of the above
- e/ a & c

Knowledge Check Q11

(non sterile section)

- Why is keeping objects dry is important in controlling microbes?
- a/ microbes can’t swim so will not down
- b/ microbes growth better when dry so a dry surface is good for them
- c/ with little food and water, microbes can flourish, increasing in number
- d/ a & b

Knowledge Check Q12

(non sterile section)

- When is a microbe a ‘bad’ microbe?
- a/ when it lurks in a cave
- b/ when we find it where we don’t want it
- c/ when it laughs maniacally
- d/ when there are too many of them in the one place
- e/ b & d

Knowledge Check Non Aseptic Answers

1: b

2: c

3: c & d

4: b

5: e

6: c

7: d

8: c

9: a, b, c & d

10: e

11: c

12: e

Knowledge Check Q1b

(sterile section)

- What are the different classes of the manufacturing environment?
- a/ Grade X, Y & Z
- b/ Grade A, B, C & D
- c/ Glass 100, 10 000 & 100 000
- d/ Class 100, 10 000 & 100 000
- e/ b & d

Knowledge Check Q2b

(sterile section)

- How can an environment be rendered sterile (and maintained as sterile)?
- a/ use radiation
- b/ throw fruit
- c/ effectively clean and sanitise the area
- d/ get a cat to lick all the surfaces clean
- e/ sterilise all incoming materials
- f/ use aseptic gowning
- g/ a, c, e & f

Knowledge Check Q3b

(sterile section)

- What are two different ways to recover microbes from a surface?
- a/ use a swab
- b/ use a tissue
- c/ use a contact (Rodac plate)
- d/ use a rescue helicopter

Knowledge Check Q4b

(sterile section)

- What are endotoxins?
- a/ poisons produced by fungi
- b/ fever causing proteins
- c/ the breakdown products of Gram negative bacteria
- d/ unacceptable in sterile injectables

Knowledge Check Q5b

(sterile section)

- How do we test for endotoxins?
- a/ we can use a LAL test
- b/ we can taste the test sample
- c/ we can use an approved and validated test for endotoxins
- d/ we can wait for an adverse patient reaction

Knowledge Check Q6b

(sterile section)

- Why must injectable drugs be free from endotoxins and microbes?
- a/ endotoxins are a problem when inside people
- b/ sterile drugs must be sterile, so free of microbes
- c/ microbes can cause infections worsening the patient (possibly killing them)
- d/ a fever can kill a patient
- e/ all of the above

Knowledge Check Q7b

(sterile section)

- What is depyrogenation? What is an example?
- a/ removing the flagella from a bacteria, e.g. a scrubbing brush
- b/ removing endotoxin from a surface, e.g. using a hot air oven
- c/ removing all pyrogens, eg autoclaving
- d/ removing all endotoxins, eg thorough washing

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Knowledge Check Q8b

(sterile section)

- What is the purpose of a media fill trial?
- a/ to fill in time on quiet days
- b/ to test the capability of the aseptic process
- c/ to give the bacteria something to do
- d/ to keep the regulators away

Knowledge Check Q9b

(sterile section)

- What is the purpose of aseptic gowning?
- a/ to protect the product from you
- b/ to protect you from the product
- c/ to make you look funny in the airlock
- d/ so we can look like ninjas

Knowledge Check Q10b

(sterile section)

- How should you behave in a clean room?
- a/ do not talk unless necessary
- b/ reduce moving around
- c/ move in a slow controlled manner
- d/ don’t lean over exposed product containers
- e/ none of the above

Knowledge Check Q11b

(sterile section)

- What might a sterility test failure indicate?
- a/ inability to fill sterile product aseptically
- b/ everything is fine in the production facility
- c/ a breakdown in the established processes
- d/ something happened at the raw material supplier
- e/ b & c
- f/ a & c

Knowledge Check Sterile Answers

1b: e

2b: g

3b: a & c

4b: b, c & d

5b: a & c

6b: c

7b: b & d

8b: b

9b: a

10b: a, b, c & d

11b: f

Conclusion

- Non aseptic

- You will now be able to identify:
  - The differences between sterile and sanitised
  - Methods used to control the numbers of microbes
  - Where one may find microbes
  - The largest source of (clean room) contamination
  - Why keeping objects dry is important in controlling microbes
  - What makes a microbe a ‘bad’ microbe

Conclusion

- Sterile Sites

- You will now be able to identify:
  - How to keep a room sterile
  - What the separate classes of rooms means
  - Why we conduct media fill trials
  - What endtoxins are and why they are bad
  - The purpose of sterility testing
  - Why the micro lab takes samples from clean rooms
  - Why aseptic gowning is required

Q&A

- Are there any questions regarding this presentation or microbiology in general?