

# A Novel Bacterial Contamination in Cell Culture Manufacturing

*Leptospira licerasiae*

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# Presentation Outline

- Objective
- How a TV Rerun Reminded of an Important Aspect of What We Do in Cell Culture Manufacturing
- Summary of Contamination
- Characteristics of the Contaminant
- Root Cause Analysis
- Impact Assessment
- Risk Control Strategies Considered
- Lessons Learned
- Acknowledgements

# Objective of this Presentation

To communicate Roche's findings associated with a novel contamination investigation at a cell culture manufacturing site

# ***House – Season 2, Episode 10***

## ***Failure to Communicate***



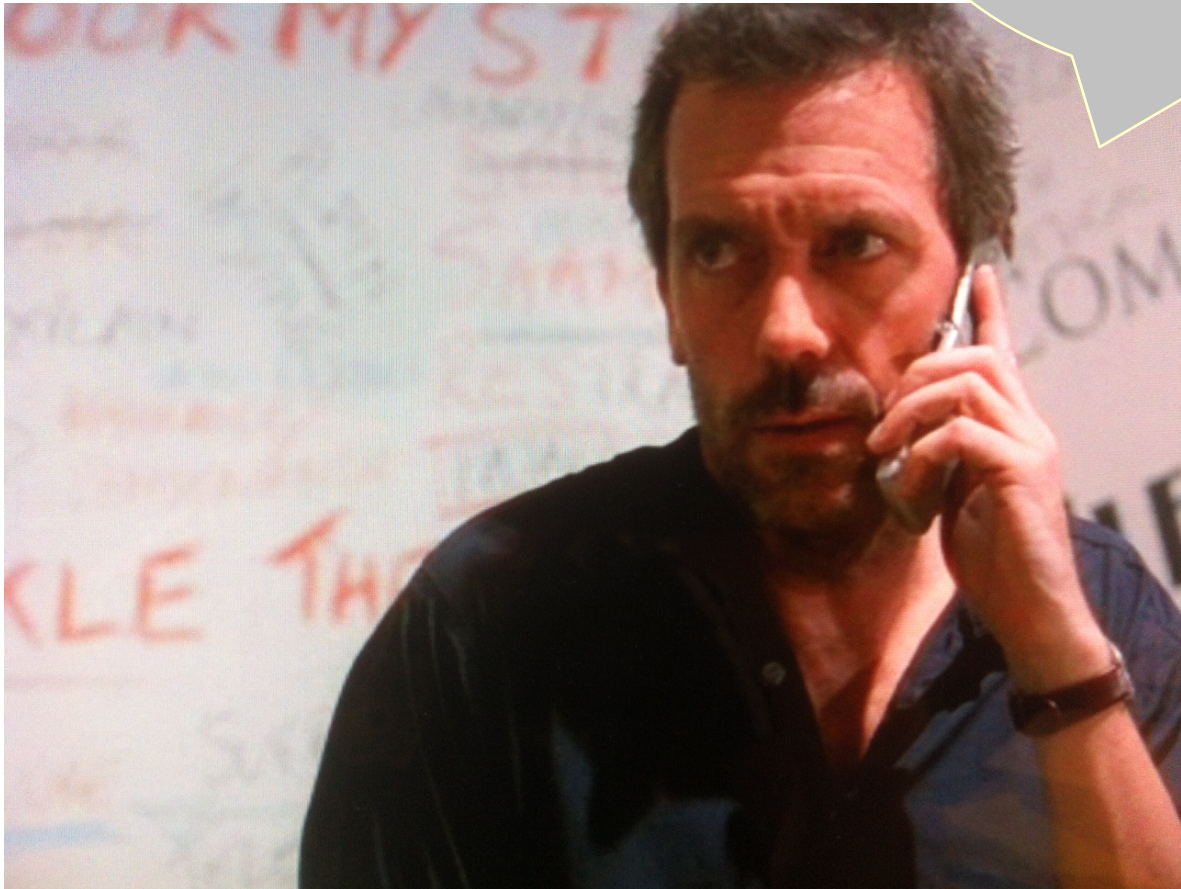
Patient in limbo – all tests give negative results, yet he continues to display strange neurological symptoms in which his speech is non-sensical



# Dr. House hypothesizes the likely cause -

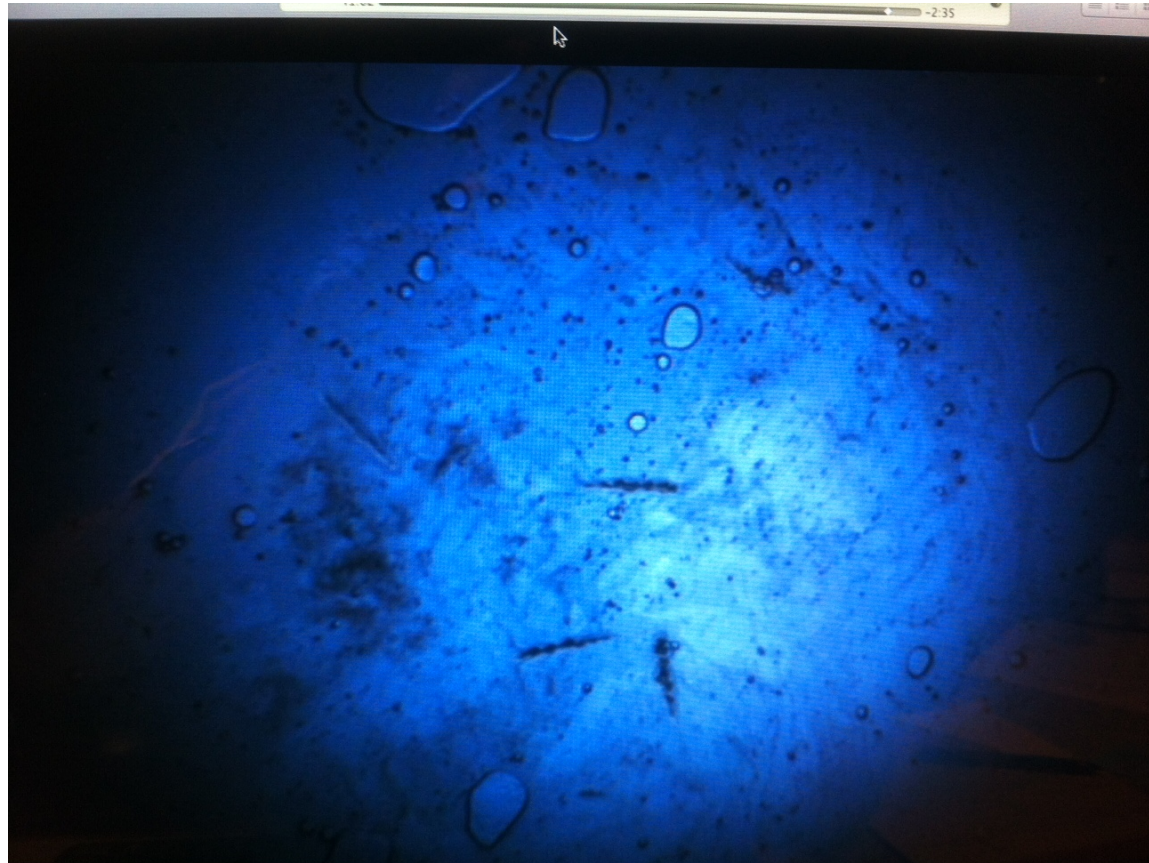


“Get his blood on a slide. And DO NOT put it through a computer this time!!”





**Finally putting the patient's sample in front of a human for analysis – cerebral Malaria is properly diagnosed**



# Dr. Eric Foreman, after finally making the diagnosis –



“If a human being had actually looked at his blood anywhere along the line, instead of just running tests through the computer, the parasites would have jumped right out at them”

# Dr. Allison Cameron responds –



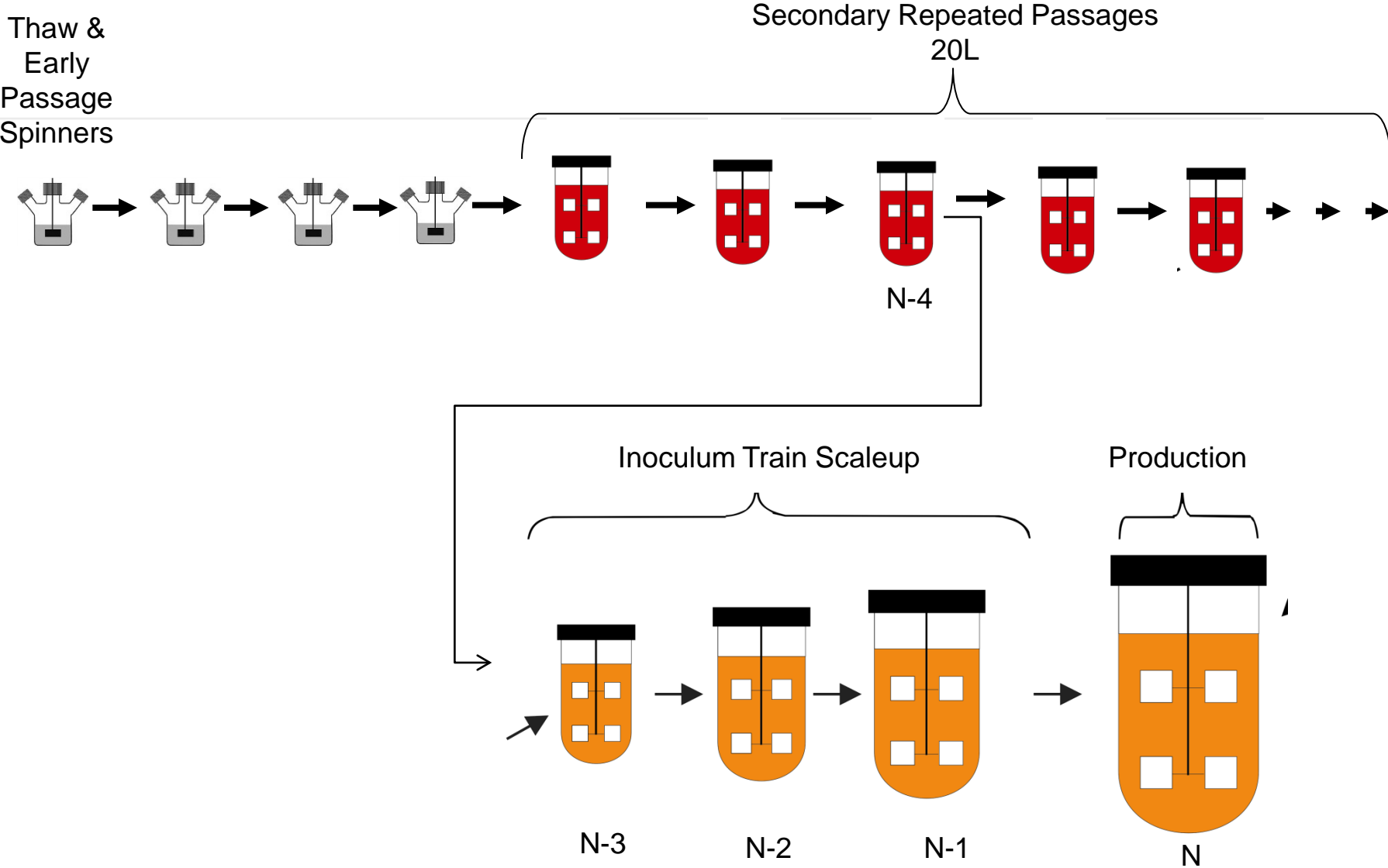
“Price of  
the  
electronic  
age...”





**Maybe we shouldn't always  
accept all elements of the  
electronic age...**

# CHO Cell Culture Process Flow Diagram



# Summary of Contamination Events

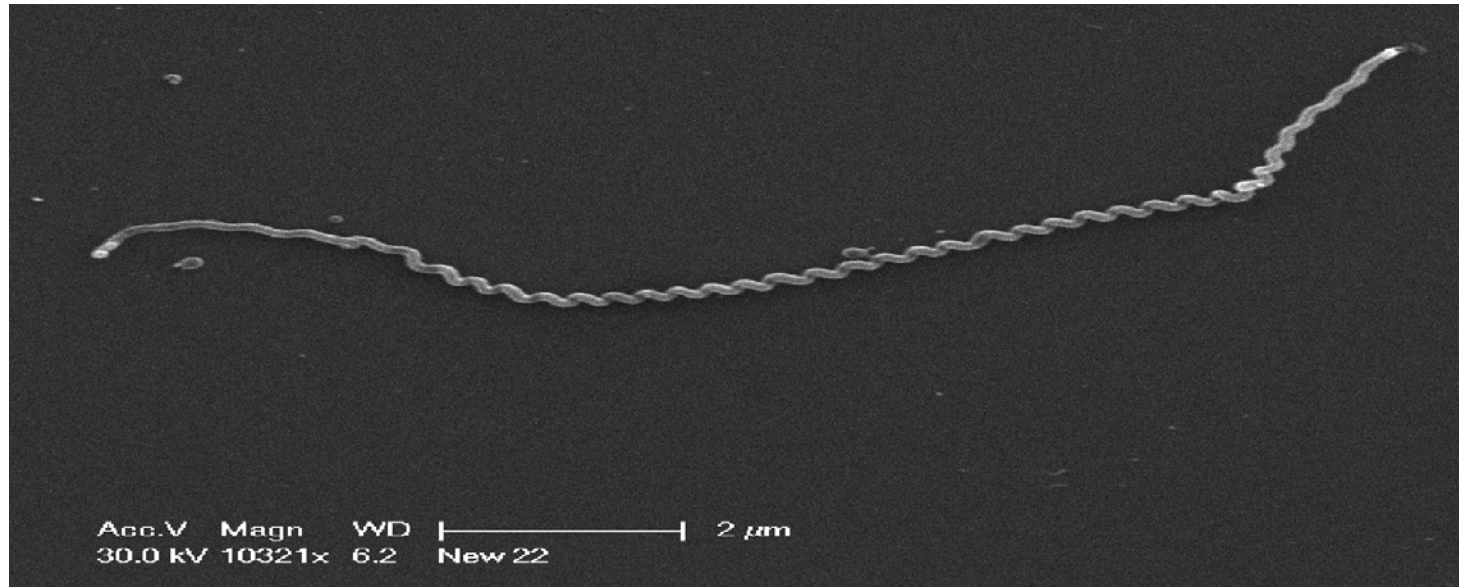
- Contamination in 20L seed train bioreactors (STB) observed during routine microscopic visual examination of the cell culture
- No bacteria observed in the Gram stain, and bioburden testing showed no growth after 5 days incubation with standard plate count media
- Bacterial DNA associated with (CHO) colonies on Blood Agar Plates purified and identified as *Leptospira licerasiae* by 16S DNA sequencing. This is a novel organism in our biological production network.
- **Neither cell culture parameters (i.e. pH, DO<sub>2</sub> and cell culture performance) nor QC standard confirmatory contamination testing detected this bacterium!**

# Summary of Contamination Events, cont'd

- Organism could not be further cultured at that time, preventing additional studies to further identify root cause
- Subsequently, a second contamination of the same organism occurred in another 20L bioreactor. This time, organism was successfully cultured, allowing additional studies to understand root cause and determine potential additional detection controls
- The scope of the events impacted multiple production batches due to the inoculation of multiple productions runs from the seed train



# Leptospira Overview

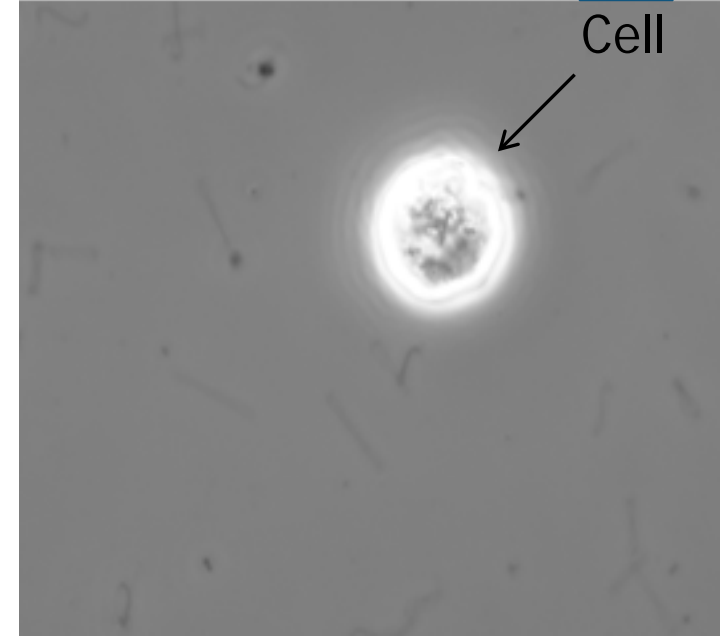


- *Leptospira* are bacteria from the Order *Spirochaetales*
- *Leptospira* are thin (0.1 μm in diameter), coiled/spiral, **motile**, cannot be visualized by the conventional bacteriological Gram staining method
- *Leptospira* are able to **survive in soil and water** for long periods of time. They have ability to form biofilms.
- **Commonly found in animals** (rodents, dogs, horses, etc.) – excreted in urine, numerous species are pathogenic, Leptospirosis is a re-emerging zoonotic disease

# Leptospira Overview

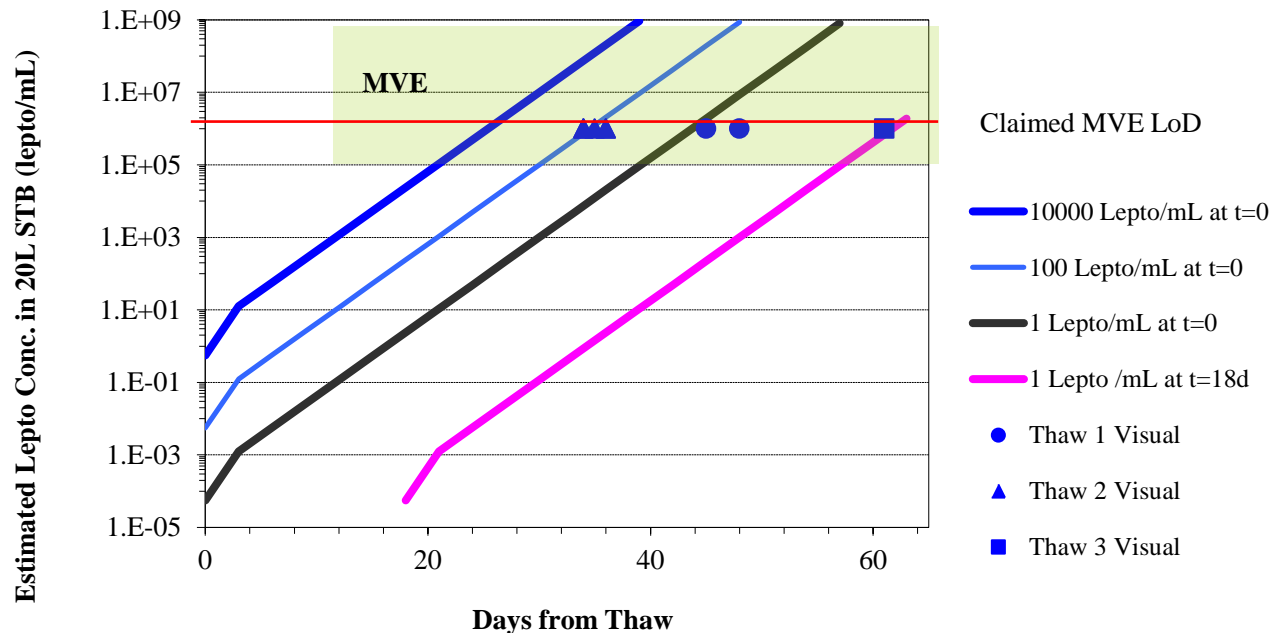


- *Leptospira* are slow-growing obligate aerobes, favor liquid environment
- Have a nutritional requirement for long-chain fatty acids – they **will not grow in typical CHO medium alone**, but will grow in the presence of CHO cells
- *Leptospira* are not spore formers and, therefore, are not expected to be heat resistant
- *Leptospira licerasiae* contains atypical lipopolysaccharides (LPS) which are weakly reactive with typical LAL methods
- *Leptospira licerasiae* possesses a gene sequence coding for hemolysin (exotoxin)
- ***Leptospira licerasiae* can pass through typical 0.1 um filters!**



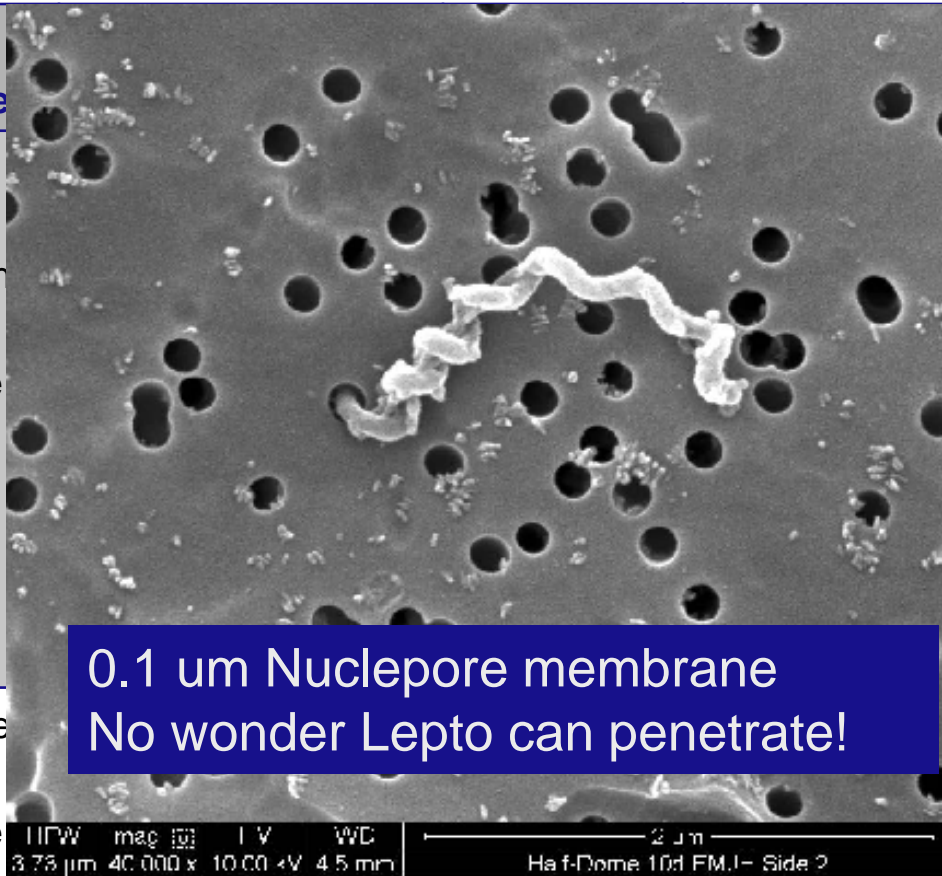
- Growth rate estimation based on laboratory co-cultivation with CHO,  $t_d \sim 16$  hr
- Event(s) genealogies and visual observation LOD suggest:
  - First event (multiple bioreactors) likely originated in spinner flasks; second event likely originated at the 20L stage
  - Initial contaminant levels estimated to be very low

Leptospira Growth Calculation (DT=16 hr)



# Initial 0.1 $\mu\text{m}$ Filtration Studies with Leptospira

Case #	Cell Source	Media growth testing
1	Lepto containing CHO culture	Leptospira observed
2		Leptospira observed
3		Leptospira observed
4		Leptospira observed
5 (positive control)		Leptospira observed
6 (negative control)	Lepto-free CHO culture	Leptospira not observed
7 (negative control)	Lepto-free CHO culture	Leptospira not observed



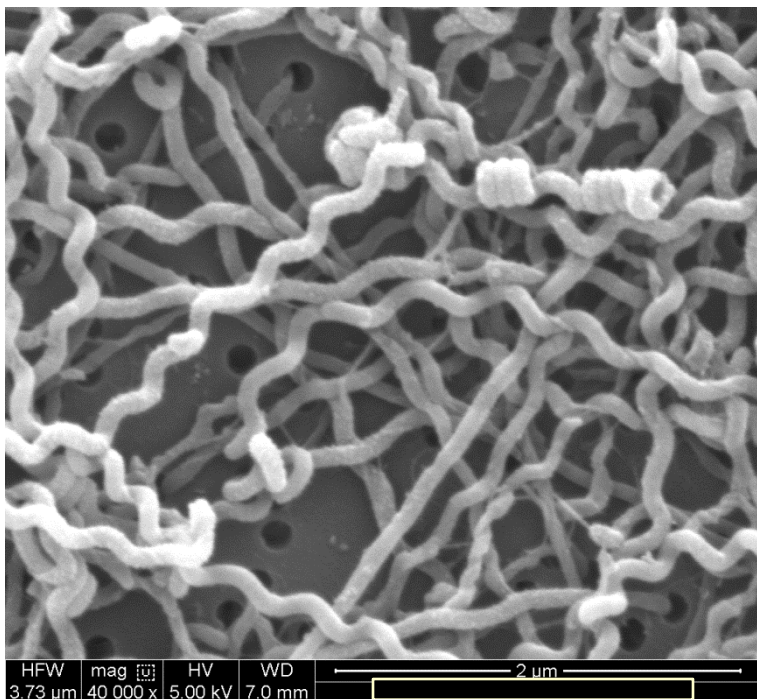
\* All cases were first filtered through 0.45  $\mu\text{m}$  PVDF to remove CHO cells

\*\* Samples were concentrated before observation. “+”, Lepto observed, “-” no Lepto observed.

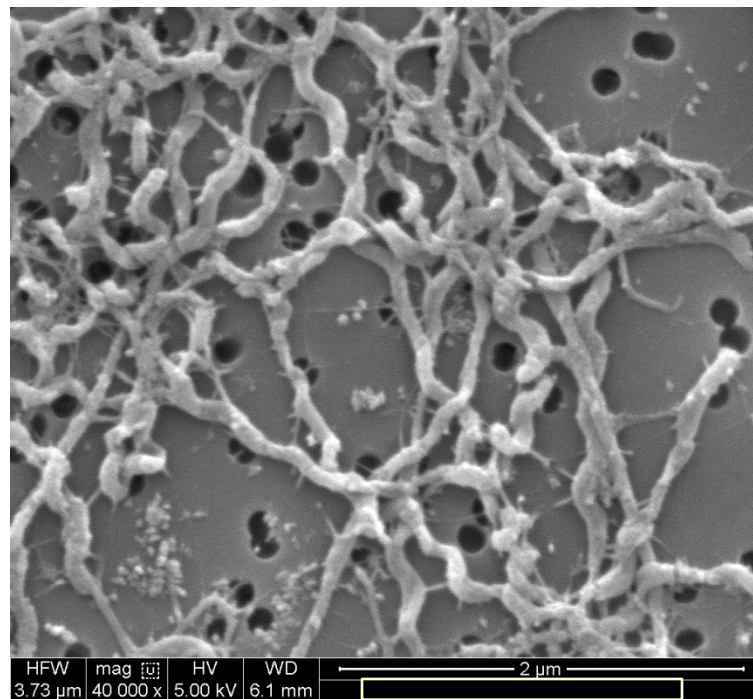


# Leptospira Morphology Dependent on Environment

- SEM images on 0.1  $\mu\text{m}$  isopore membrane filters



Cultured 10 days in EMJH



Cultured 7 days in EMJH,  
3 days in Product C medium

# Investigation Actions Associated with Events

- Successfully cultured *L. licerasiae* in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium, enabling root cause investigation and detection methods evaluation
- Implemented non-routine culture testing in EMJH medium to enhance detection in the following samples:
  - Aliquot from each Working Cell Bank (WCB) ampoule thaw
  - Pre-harvest Cell Culture Fluid (PHCCF)
- Optimized and implemented a commercial *Leptospira*-specific PCR assay to enhance detection sensitivity. Estimated LOD to be 100 organisms/mL in PHCCF.

# Investigation Actions Associated with Events



- Estimated the LOD of microscopic visual examination to be  $10^6$  organisms/mL
- Estimated *Leptospira licerasiae* doubling time in CHO cell culture to be  $\geq 16$  hours
- Performed survey testing across the manufacturing network using PCR method to further confirm the scope of investigation – **none detected**
- Performed global risk assessment of current upstream microbial control system in relation to this novel microorganism and root cause analysis

# Global Risk Assessment



- Fault Tree Analysis (FTA) was the risk assessment methodology
  - The failure was defined as “potential contamination of cell culture production with microorganisms that have limited detectability with the current control systems (*L. licerasiae* as a worst-case model)”. The FTA evaluated the following six categories of potential failure pathways:
    - Raw material, Process, Equipment, Personnel, Environment, and Potential weaknesses in the current detection systems.
    - A total of 101 potential failure points were identified and evaluated; numerous actions were taken and activities performed based on this evaluation.
  - The current prevention and detection control systems were evaluated for all identified failure points with respect to their adequacy and the need for any additional controls. Recommendations for improvements were identified as an output of the Fault Tree Analysis.



# Root Cause Analysis



- The most probable root cause for the contaminations was determined to be the small volume media preparation process occurring in the Small Volume Media Preparation Area:
  - Based on PCR results, EMJH medium culture study results, and data review, it was concluded that low levels of contaminations likely occurred very early in the seed train
  - *Leptospira licerasiae* mostly likely present in the pre-filtered small volume media since only single-organism contaminations were observed, and demonstrated that *L. licerasiae* can pass through 0.1  $\mu\text{m}$  filters
- Potential source of *L. licerasiae*??

# Root Cause Analysis, cont' d



- Potential source of *L. licerasiae*
  - Raw Materials – no evidence found, but very difficult to test conclusively
  - Environment (found spirochetes in untreated water source used in site cooling tower) – **Discontinued use of this water source**
  - Personnel – no evidence found, but personnel could be carrier from environment
- Based on extensive testing (EMJH / PCR), concluded that the working cell bank is very unlikely to be the source of *Leptospira licerasiae*

# Impact Assessment



- Product
  - pH inactivation
  - Virus filtration
  - Impurities clearance (endotoxin, exotoxin, etc)
  - Drug Substance freeze/thaw process
- Equipment and Facilities
  - pH inactivation – cleaning effectiveness
  - Heat inactivation – cleaning and sanitization
  - Disinfectant efficacy testing – environmental control

# pH Inactivation studies



- *Leptospira licerasiae* at  $10^4$ /mL and  $10^8$ /mL exposed to differing pH and exposure times in conditioned affinity pool
- Treated samples incubated in EMJH cultural test method

pH	Time (min)	Result
2.8	15 to 240	No growth observed for all exposure times
3.4	15 to 240	No growth observed for all exposure times
3.6	15 to 240	No growth observed for all exposure times
4.0	15 to 240	<b>No growth observed for all exposure times</b>
5.0	15 to 240	At $10^4$ /mL – Growth at 15 min exposure; No growth for all exposures $\geq 30$ min At $10^8$ /mL – Growth at up to 60 min exposure; No growth for all exposures $\geq 120$ min
6.0	15 to 240	Growth observed under all exposure times

- Typical low-pH hold step ( $<4$ ) for Mab production effective at inactivation should a contamination go undetected in cell culture



# Heat Inactivation Studies



- *L. licerasiae* at  $\geq 10^6$ /mL, Treated samples incubated in EMJH cultural test method

## Lab-Scale Batch Exposure

Temp (C)	Time (sec)	Result (Growth / No Growth)
45	5, 10, 30, 60	Growth
65	5, 10, 30, 60	No Growth
85	5, 10, 30, 60	No Growth
100	5, 10, 30, 60	No Growth

## Pilot-Scale Continuous Flow Exposure (HTST)

Temp (C)	Time (sec)	Result (Growth / No Growth)
65	5, 10, 20	No Growth
102 (std media HTST)	5, 10, 20	No Growth

- Heat treatment at mild conditions very effective at inactivation
- Provides good assurance that the organism is unlikely to persist in process equipment or the facility (cleaning and sanitization efficacy)

# Filtration Removal Studies



Four “typical” 0.1 um sterilizing-grade filters failed to retain *Leptospira*!

Filter Type	Result
0.1 um sterilizing-grade Product A	Not retentive
0.1 um sterilizing-grade Product B	Not retentive
0.1 um sterilizing-grade Product C	Not retentive
0.1 um sterilizing-grade Product D	Not retentive
0.1 um sterilizing-grade Product E (designed for improved mycoplasma removal)	Retentive under v <sub>max</sub> , but not p <sub>max</sub> , conditions (=> not robust)
Large virus removal filter Product F	Retentive
Large virus removal filter Product G	Retentive

- Both large virus removal filters retained *L. licerasiae*. These are not likely feasible for media filtration, but indicate significant downstream clearance should a contamination go undetected in cell culture

# Risk Control Strategies Considered



- **Prevention**

- Upstream prevention (media prep procedures)
- Barriers to prevent entry of adventitious agents into process streams
  - Heat treatment
  - Filtration
  - UV-C



- **Detection**

- EMJH media culture testing for non-frozen pre-harvest cell culture fluid samples and for residual fluid from each Working Cell Bank ampoule
- PCR testing on pre-harvest cell culture fluid samples across the network

# Lessons Learned



- *L. licerasiae* is a bacterium that can **pass through industry-standard 0.1um filters** and contaminate a CHO culture with **no direct evidence of its presence!**
- Extend risk assessment to other potential sources of contaminants with similar characteristics, and **update control strategies based on new information** – Enhanced upstream barriers may be warranted (e.g., heat treatment or other virus barriers)
- Spirochetes are in the environment; current compendial test methods are **not able to detect**

# Lessons Learned, cont' d

- **Look at your cell cultures** – Had routine microscopic examination not been in place, the contaminations may have gone undetected!
- Engaging in **timely communications** with Health Authorities and Commercial Partners is critical
- Sharing this knowledge (lessons learned) with the industry is **important for patients and for the industry** to continually improve control mechanisms

# Acknowledgements (and many more...)



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**Bold** indicates presentation preparation