

The Value of Postmortem Microbiology Cultures

Stefan Riedel, MD, PhD, D(ABMM)

Associate Professor, Pathology

The Johns Hopkins University, School of Medicine
Department of Pathology – Division of Microbiology

Director Clinical Pathology Laboratories

Johns Hopkins Bayview Medical Center



Conflict of Interest / Disclosures

Stefan Riedel, M.D., Ph.D.

“Nothing to disclose”

Learning Objectives

1. Describe and understand the potential concerns regarding the postmortem spread of microorganisms.
2. Describe and understand the methods for specimen procurement for postmortem microbiology cultures.
3. Describe and understand the limitations of postmortem microbiology cultures and examinations.

Some Facts to Consider

Infections are among the 15 leading causes of death in the U.S.

- Influenza & pneumonia are ranked 8th
- Septicemia is ranked 11th
- Significant problems with high attributable cost
- Rising antimicrobial resistance

Hoyert DL, Xu J. 2012. *Natl Vital Stat Rep*; 61: 1-52

Interest in postmortem microbiology dates back in time !

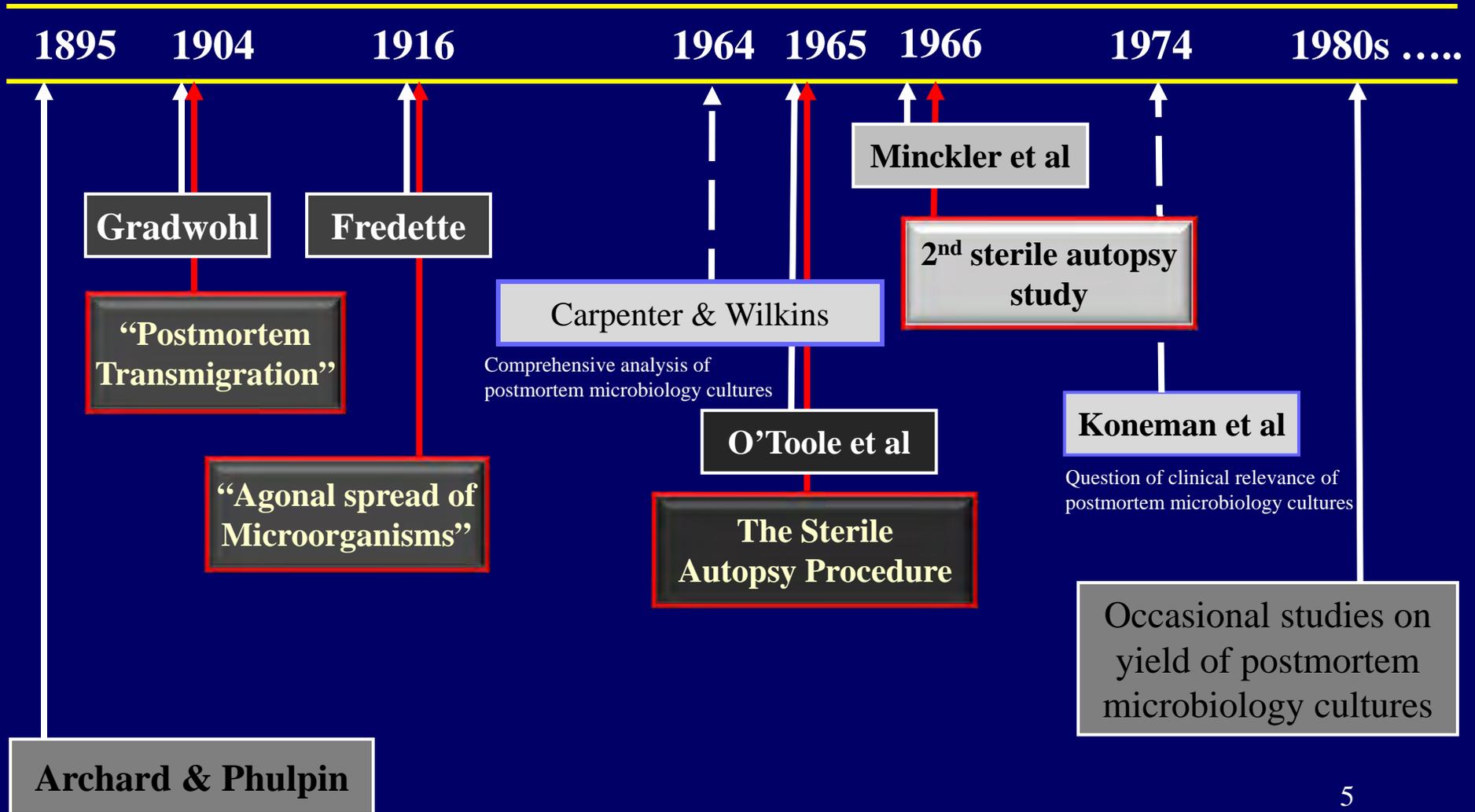
- Contributed to better understanding of infectious diseases
- Contributed to discovery of pathogenic microorganisms
- Value for forensic autopsies and medicolegal investigations
- Diagnostic & epidemiologic tool in disease outbreak situations

Autopsy rates continuously declined since the 1950s

Lack of specific guidelines or recommendations by regulatory agencies

Postmortem Examination & Microbiology

A Brief Perspective of History



The meaning of a positive culture result

**Genuine positive bacterial culture
indicates infection**

Culture contamination ?



Postmortem Transmigration of Bacteria



Agonal Spread of Bacteria

Postmortem Transmigration

Importance de l'Examen bactériologique

PRATIQUÉ SUR LES CADAVRES

PAR LE D^r R. B. H. GRADWOHL

Instructeur d'anatomie pathologique à l'Université.
Médecin attaché au Parquet de Saint-Louis (Missouri) E. U. A.

R.B. Gradwohl – 1904

Ann. Inst. Pasteur (Paris) 18: 767-773

“Bacteria migrate from mucosal surfaces into the bloodstream after the circulation has ceased, i.e. after death.”

Postmortem Transmigration : more support ?

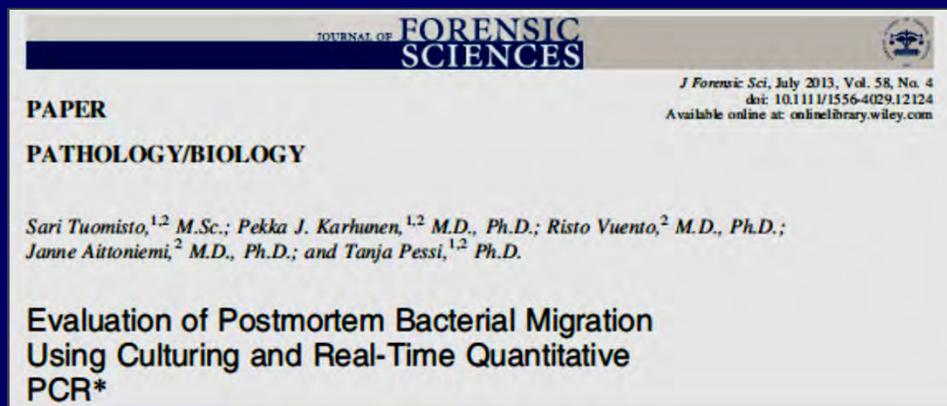
1. Epstein EZ, et al. 1929. *J. Infect. Dis.* 44: 327-334
2. Carpenter HM, et al. 1964. *Arch. Pathol.* 77: 73-81
3. Rose GW, et al. 1971. *Am. Health Lab. Sci.* 8: 75-78

- Reviews of “clinical” autopsy cases
- Culture positivity increased from 20% to 40% correlating to the length of the postmortem interval²
- Positivity of lung cultures increased in relation to hospital LOS and postmortem interval²
- Study results verified the proposed concept of transmigration

- a. Newsom IE, et al. 1929. *J. Infect. Dis.* 45: 386-392
- b. Burn CC. 1934. *J. Infect Dis.* 54: 388-394
- c. Kellerman GD, et al. 1976. *Am. J. Clin. Pathol.* 66: 911-915

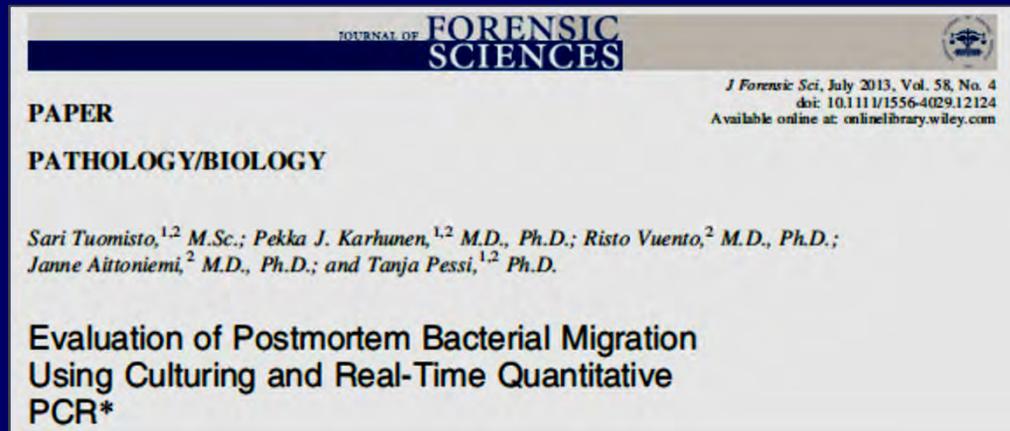
- In-vitro & animal studies
- Transmigration of bacteria through intact intestinal wall in humans within 12-15 hours^c
- Study results were in support of “clinical evidence” of the proposed concept of transmigration

Postmortem Transmigration : current support?



- Study design:
 - Prospective ; 33 consecutive cases (forensic autopsies)
 - Out-of-hospital death; male gender; age > 18 years
 - Time interval between death and storage of body in morgue : < 24 hours
 - Time elapsed postmortem : < 7 days
 - No indication of infection; no antibiotic therapy 2 weeks prior to death
- Use of culture and PCR over period of 5 days postmortem
- Bacteria migrate postmortem from intestine to blood, liver, portal vein, mesenteric lymphnodes, and pericardium
- Other potential sources of migration: skin, oral cavity, respiratory tract
- PCR detected more organism throughout entire postmortem interval than culture
- Sterility by culture method: pericardial fluid (94%) ; liver (64%) ; blood (40%)
- Cumulative sterility by PCR: 42% (1-3 days); 38% (4-5 days); 24% (> 5 days)

Postmortem Transmigration : current support?



Postmortem Transmigration is real !



Blood cultures are unreliable specimens for postmortem cultures
(high rate of contamination and polymicrobial, enteric organisms)



Liver and pericardium are most suitable for sampling
(higher and more stable rates of sterility)

Agonal Spread of Bacteria

J.W. Fredette – 1916

Invasion of bacteria into the bloodstream when systemic circulation is declining during the agonal period or when artificially maintained during resuscitation measures.

The concept of bacteremias in the agonal period : “terminal infection”

- Bacterial invasion into the bloodstream is spontaneous, in the absence of “organic destruction of tissue surfaces”
- Invasion arises in areas which naturally harbor the organism(s)

The study

- Surgical preparation of the median cephalic vein and application of iodine
- Tip of sterile sealed capillary was broken, flamed, and inserted to vein
- At least 1 mL of blood collected into the capillary, followed by inoculation onto standard culture media for 24-36 h of incubation

Fredette J.W. 1916. Bacteremias in the agonal period. *J. Lab. Clin. Med.* 2: 180-188

- 119 cases included in study; 77 negative cultures & 42 positive cultures
- 31/42 positive cultures were monomicrobial (74%); 11/42 had two organisms (26%)
- Streptococci were most frequent “terminal invaders”
- Only 14 antemortem cultures were collected; 8 showed no growth, with 4 showing ‘*no growth*’ on postmortem culture

1. Routine blood cultures taken immediately after death reveal the presence of an unsuspected bacteremia in about one-third of all fatal cases.
2. Streptococci are the most frequent terminal bacterial invaders of the blood stream.
3. Bacteriological findings at autopsy within a few hours after death, though fairly reliable in demonstrating the presence of organisms existing at the time of death, do not exclude the possibility of postmortem invasion.
4. The taking of frequent antemortem, immediate postmortem, and autopsy cultures is to be encouraged. Contamination may be obviated by a simple technic.
5. In the absence of adequate autopsy material, the routine taking of immediate postmortem cultures will furnish valuable information as regards the essential and terminal bacteremias.

Fredette J.W. 1916. Bacteremias in the agonal period. *J. Lab. Clin. Med.* 2: 180-188

110 cases included in study: 77 negative cultures & 42 positive cultures

*“...bacteriological findings before and after death would be particularly interesting, in view of the fact that similar results would confirm the provisional antemortem bacteriological diagnosis, while the finding of additional organisms would more firmly establish the more or less theoretical supposition of “agonal” or terminal infection...
....Unquestionably, bacteremias, and oftentimes fatal ones, do exist where they are unsuspected.However, it is not our purpose to discuss those bacteremias existing for a considerable time before death.”*



Lex parsimoniae
(Occam's razor)

Occam's razor doesn't necessarily go with the simplest theory, whether it's right or wrong; it merely tries to cut through the clutter to find the best theory based on the best scientific principles and knowledge at the time. While Occam's razor is a useful tool, it has been known to obstruct scientific progress at times.

AAAS online; June 12, 2012

postmortem cultures will furnish valuable information as regards the essential and terminal bacteremias.

**Fredette J.W. 1916. Bacteremias in the agonal period.
J. Lab. Clin. Med. 2: 180-188**

“...bacteriological findings before and after death would be particularly interesting, in view of the fact that similar results would confirm the provisional antemortem bacteriological diagnosis, while the finding of additional organisms would more firmly establish the more or less theoretical supposition of “agonal” or terminal infection...Unquestionably, bacteremias, and oftentimes fatal ones, do exist where they are unsuspected.However, it is not our purpose to discuss those bacteremias existing for a considerable time before death.”



Antemortem culture results and clinical context were not considered as significant part of the evaluation of autopsy results



Approach to culture and organism identification was limited by the available methods of that time



Incomplete understanding of the definition and process of bacteremia / sepsis compared to today's standards

Agonal Spread – additional supportive evidence ?

Point

Koneman EW, Davis MA. 1974. *Am. J. Clin. Pathol.* 61: 28-40

- 396 autopsies; performed within 6 h of death
- Tissue cultures and blood cultures were obtained
- 170/321 postmortem heart blood cultures were positive (53%); 74% were monomicrobial
- Up to 70% of lung tissue biopsies were positive for a potential pathogen
- High level (52%) of disagreement between antemortem and postmortem cultures, suggestive of agonal invasion
- Authors quote experimental studies to support the concept of tissues suffering “a lowering of vitality” and loss of “resistance to withhold migration of bacteria into the body during the agonal period”

Counterpoint

Smith RF, et al. 1975. *Am. J. Clin. Pathol.* 63 (4): 502-508

- Prospective study in 20 children who died from complications of thermal injuries
- Various typical organisms isolates in antemortem and postmortem cultures (incl. *S. aureus*, *P. aeruginosa*)
- Results from postmortem microbiologic examination correlated “reasonably well” with antemortem cultures
- Redefinition of some cases previously to have had been described as “terminal sepsis”

The Value of Two Theoretical Concepts

Agonal Spread

vs.

Postmortem Transmigration

Bacteria invade bloodstream during the time of dying.

- theoretical in concept
- little “clinical” and/or scientific evidence
- scant support in “recent” literature (1974/1975)
- too few cases
- retrospective, observational study design
- concept of “terminal sepsis” ?



Bacteria invade bloodstream after death but before autopsy.

- supported by in-vitro experiments
- some “clinical” and/or scientific evidence
- some support in recent literature (1960s to 2011)
- retrospective, observational studies with sufficient statistical power
- few prospective studies, utilizing appropriate laboratory detection methods



Postmortem Transmigration is still commonly accepted concept by pathologists & microbiologists !

Practical & Theoretical Aspects of Autopsy in the Context of Postmortem Spread of Organisms

Multiplication of organisms in the body after death depends on

Organism's rate of division ; Supply of nutrients
Concentration of oxygen ; "Ambient" temperature

Postmortem drainage of saliva into lung in at least 50% of patients

Norris C, Pappenheimer AM. 1905. *J Experiment Med* ; 7: 450-472

Experimental passage of organism through human intestinal wall
within 12-15 hours after death

Kellerman GD et al. 1976. *Am J Clin Pathol* ; 66: 911-915

Cooling of body postmortem limits bacterial growth

Roberts FJ. 1998. *Eur J Clin Microbiol Infect Dis*; 17: 821-827

A few other (historic) reviews of postmortem microbiology

Roberts FJ. *Canad Med Assoc J* 1969; 100: 70-74

- prospective evaluation of 100 autopsies (clinical information, antemortem & postmortem cultures)
- conditions to be evaluated: bacteremia, peritonitis, pneumonia
- cultures obtained from: heart, peripheral blood, liver, spleen, and both lungs
- organ surfaces were seared to dryness with an electric soldering iron
- tissue cultures were collected by forcing a sterile cotton swab deeply through the seared area
- swabs cultured on sheep blood agar, MacConkey agar, and trypticase soy broth

- collection of 5 routine cultures recommended to detect undiagnosed bacteremia
- spleen is most reliable site for detection of bacteremia
- postmortem lung cultures are not reliable to detect pneumonia due to contamination with upper respiratory flora (utility in very select cases)

Washington JA, et al. *Arch Path* 1972; 94: 245-249

- human tissues are not necessarily sterile at any given point of time
- discordance between antemortem and postmortem culture results
- usefulness of postmortem bacteriologic studies is limited, except in select cases
- “postmortem microbiologic assessment” of tissue used for organ transplantation is warranted

A few other (historic) reviews of postmortem microbiology

du Moulin GC, et al. *Clin Microbiol Newsletter* 1988; 10 (21): 165-167

- review of current evidence
- postmortem bacteriologic studies are useful and an important part of postmortem examinations
- correlation with histologic pathologic findings and antemortem clinical information is imperative
- potential aid in discovery of unknown causes of infections

Wilson SJ, et al. *Arch Pathol Lab Med* 1993; 117: 986-988

- Comparison of postmortem BCs in 111 autopsies with antemortem BCs
- 54% positive postmortem cultures despite COD unrelated to infection
- 76% of cases with negative antemortem cultures had postmortem cultures with contaminants
- Postmortem cultures (using traditional methods) have little to no diagnostic utility
- Considering fiscal concerns in healthcare, postmortem cultures should only be obtained if no antemortem cultures are available
- Results must be interpreted with caution (correlation with gross & microscopic pathologic findings)

Limitations of the *Historic Autopsy Studies*



Lack of standardization for sample procurement and culture procedure



Continuously monitoring blood culture systems were not available



Often too few cases per study



No “gold standard” for defining existing, antemortem, underlying infectious disease

Factors Influencing the Value of Autopsy Microbiology



Minimizing contamination of cultures obtained in the autopsy room !



What is the indication to obtain cultures ?



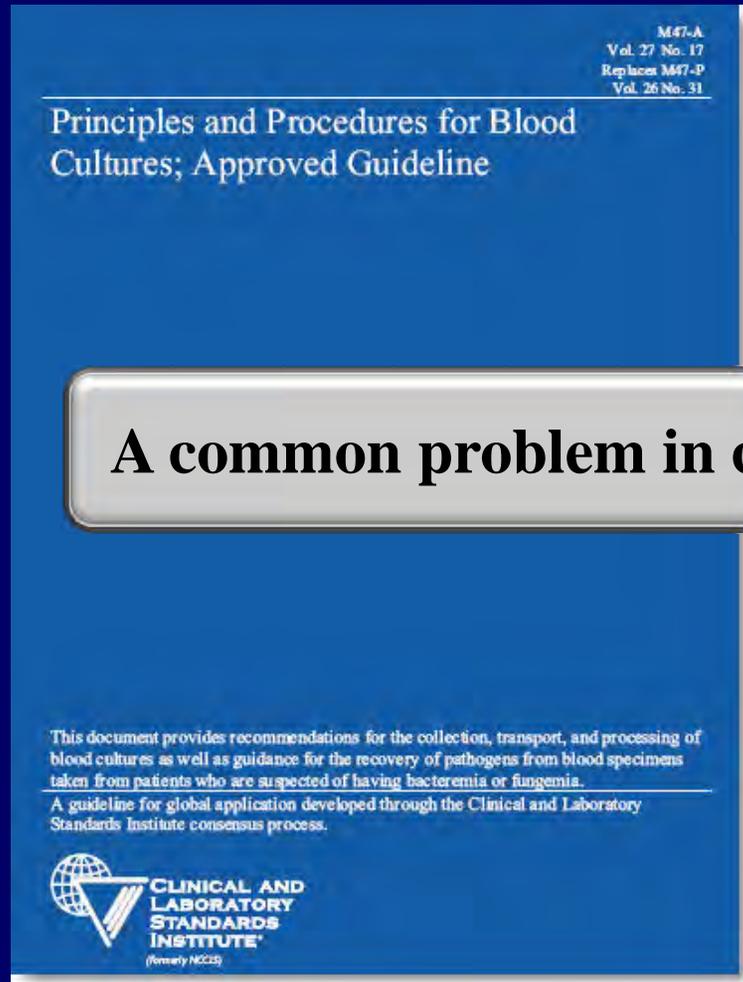
Selecting the most appropriate specimen and approach to procurement !



What is the postmortem interval to autopsy?

Contamination of Microbiology Cultures

Example : blood cultures



A common problem in clinical microbiology cultures

Blood Culture Contamination in the Clinical Laboratory

- Definition & Algorithms

- Typical skin pathogens
 - viridans strep, *Corynebacterium* sp., *Bacillus* sp., coagulase negative staphylococci, *P. acnes*
- Number of positive and negative cultures in an episode
- Results of concurrent microbiology tests
- Compatibility of clinical features with typical features of infection
- Rates should be < 3%

Nearly always (>90%) causes of bacteremia:

Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Candida albicans

Almost never causes of bacteremia:

Corynebacterium spp, *Bacillus* spp (not *B. anthracis*),
Propionibacterium acnes

The “Sterile Autopsy”

O’Toole WF, et al. 1965. *Arch Pathol*; 80: 540-547

Minckler TM, et al. 1966. *Am J Clin Pathol*; 45: 85-92

- Introduction of tissue procurement centers with “sterile morgue facilities” allowed for re-evaluation of postmortem microbiology procedures
- 54 autopsy cases with 440 cultures
- Surgical cleaning of the body
- Autopsy staff undertook “surgical scrub & gowning”
- Dissection was performed in autopsy room with controlled air flow and use of sterile, surgical instruments
- Postmortem interval < 20 hours
- Samples were cultured on standard microbiology media
- No antemortem suspicion or evidence of infection

The “Sterile Autopsy”

- no growth in 25 cases
- bacterial growth in 29 cases, but mostly contaminant organisms
- 324/440 samples (74%) no growth
- 12/47 samples from spleen had growth; 6 were pathogens

O’Toole WF, et al. 1965. *Arch Pathol*; 80: 540-547

- 262 surgeries and 213 autopsies
- 263 tissue samples during surgery; 114 from GI tract (showing intestinal resident flora)
- 485/738 tissue samples from autopsies were sterile (66%)
- 12/47 samples from spleen had growth; 6 were pathogens

Minckler TM, et al. 1966. *Am J Clin Pathol*; 45: 85-92



Sterile / aseptic procedures can prevent contamination

Cultures positive with typical pathogens likely
represent true infection

Minimizing Contamination in the Autopsy Room

Body should be moved to a 4°C to 6°C refrigerated locker as soon after death as possible

Avoid unnecessary movement of body

Clean autopsy room with good ventilation
(at least 20 air changes per hour)

Consider autopsy to be performed as a sterile procedure (see O'Toole and Minckler)

“What to do if infection is considered”

Infectious diseases

A significant cause of morbidity & mortality

Hoyert DL, Xu J. 2012. *Natl Vital Stat Rep*; 61: 1-52

The assessment of the significance of bacterial culture results in life as well as in death depends on the specific isolate and the clinical context !

- Consider major clinical syndromes
 - Respiratory tract infections / pneumonia
 - Septicemia
 - Central nervous system infections (meningitis, encephalitis)
 - Fever / rash
- Consider reason for & length of hospitalization prior to death
 - Nosocomial infection ?
 - Immune status ?
 - Treatment failure ?
- Consider epidemiologic setting in case of illness
 - Nosocomial infection ?
 - Considerations for potential disease outbreaks

Things to consider when deciding to perform postmortem microbiology

- Select appropriate type of culture:
 - Standard bacteriology (most frequently performed)
 - Viral cultures
 - Mycobacterial cultures
 - Fungal cultures
- Decision to submit tissue/fluid for culture should consider results from visual inspection of body and initial gross exam prior to evisceration
- Specimens considered for culture:
 - Blood
 - Tissue
 - Urine
 - CSF
- Use different, sterile instruments for each culture site, if multiple cultures are collected from different sites

Procurement of Specimens

Some Things to Consider

The greatest concerns: contamination & procurement of a representative sample !

**Blood and tissue cultures should be collected
within 24 to 48 h of death**

➔ Collect samples **PRIOR** to
evisceration of body

➔ Use of sterile / aseptic
technique for sample
collection



Blood and other fluid samples should be collected FIRST !

Blood Cultures

Heart blood, spleen, peripheral venous blood (femoral, external iliac, subclavian veins)



Positivity Rate : 20% - 69% (postmortem) vs. 0-25% (antemortem)

- contamination, differences in patient populations, (“agonal bacteremia”)
- few antemortem cultures are obtained during the last hours of life
- in true bacteremias/sepsis most cultures will yield the same growth



A typical pathogenic organism in monomicrobial culture is likely a TRUE pathogen !

Peripheral venous blood collection

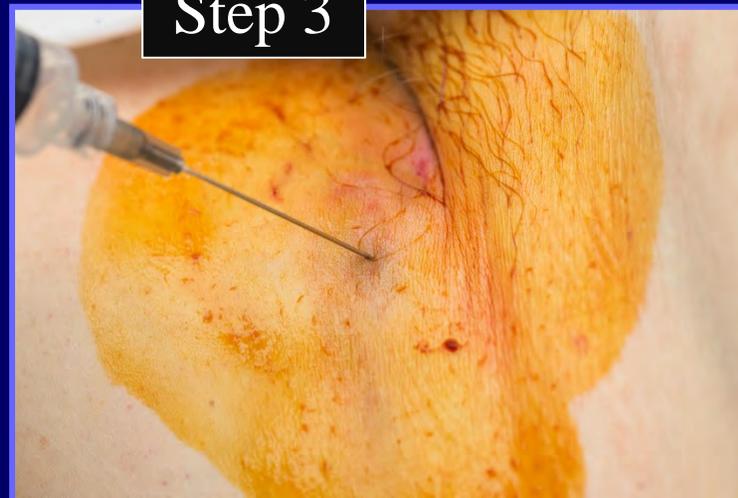
Step 1



Step 2



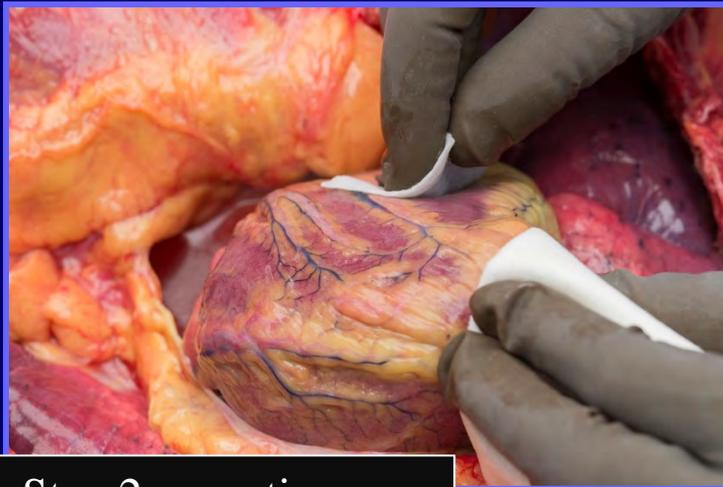
Step 3



Images: Courtesy of J. Hooper, MD;
JHU Autopsy Pathology

Heart blood for culture

Step 1 : removal of pericardial fat and location of heart

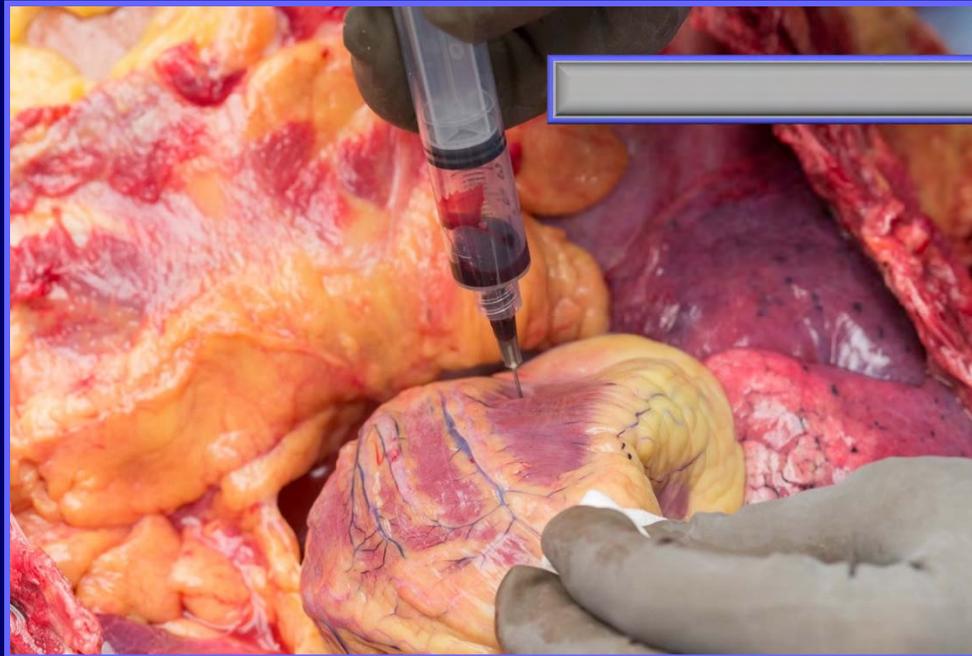


Step 2 : aseptic prep



Step 3 : collection of blood

Heart blood for culture



Obtain 10-20 mL of blood & transfer to BC bottles

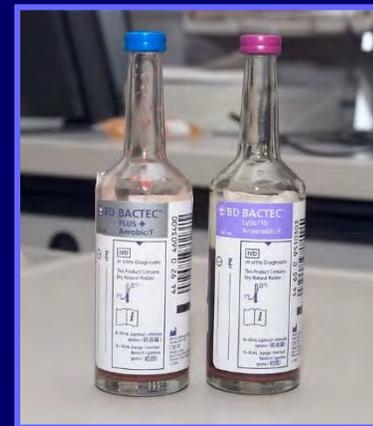


Image: Courtesy of J. Hooper, MD;
JHU Autopsy Pathology

- Transfer to BC bottles (1 set: aerobic & anaerobic BC bottle)
- Incubation in continuous monitoring blood culture technology
- Follow usual (clinical) protocol [5 day incubation; if negative at that point, discard]
- Avoid contamination during transfer

Postmortem blood isolates likely to be true positives

- *Neisseria meningitidis*
- *Haemophilus influenzae*
- *Streptococcus pneumoniae*
- *Staphylococcus aureus* (MSSA, MRSA)
- *Salmonella* species
- *Escherichia coli*
- other (clinically significant) Enterobacteriaceae
 - *K. pneumoniae*, *Enterobacter* spp.
- *Candida albicans* (consider clinical setting)



Consider antimicrobial resistance of isolates in the appropriate clinical context

Considerations for Tissue Cultures

- Lung, liver, kidney, bone-marrow
- Lower respiratory tract usually a sterile body site

Lung sections are frequently obtained during postmortem examination.



Probably most controversial of specimens !

- Obtain biopsies *in-situ*, prior to evisceration
- Organ surface should be sterilized (searing surface with hot spatula or alcohol swab)
- Use of sterile instruments to obtain tissue biopsies



Despite strict aseptic technique , lung tissue biopsies are frequently prone to contamination !

Collection of Lung Tissue



Images: Courtesy of J. Hooper, MD;
JHU Autopsy Pathology

Transport tissue specimens in tubes containing transport media or sterile broth. Process specimens asap; otherwise refrigerate.

Lung Cultures: *Potential Problems*

1. Correlation of culture sites / results with that of anatomical specimens / microscopic examination
2. Extension of respiratory flora into lower respiratory tract
3. Drainage of saliva into lungs after death (what is the postmortem interval?)

Lung cultures may be positive
even in the absence of histologic evidence of pneumonia !

Norris C, Pappenheimer AM. 1905. *J Experiment Med* ; 7: 450-472
Roberts FJ. 1969. *J Canad Med Assoc* ; 100: 70-74
Zanen-Lim OG, et al. 1980. *J Clin Pathol*; 33: 474-480



Accuracy of postmortem lung cultures may equal that of expectorated sputum !



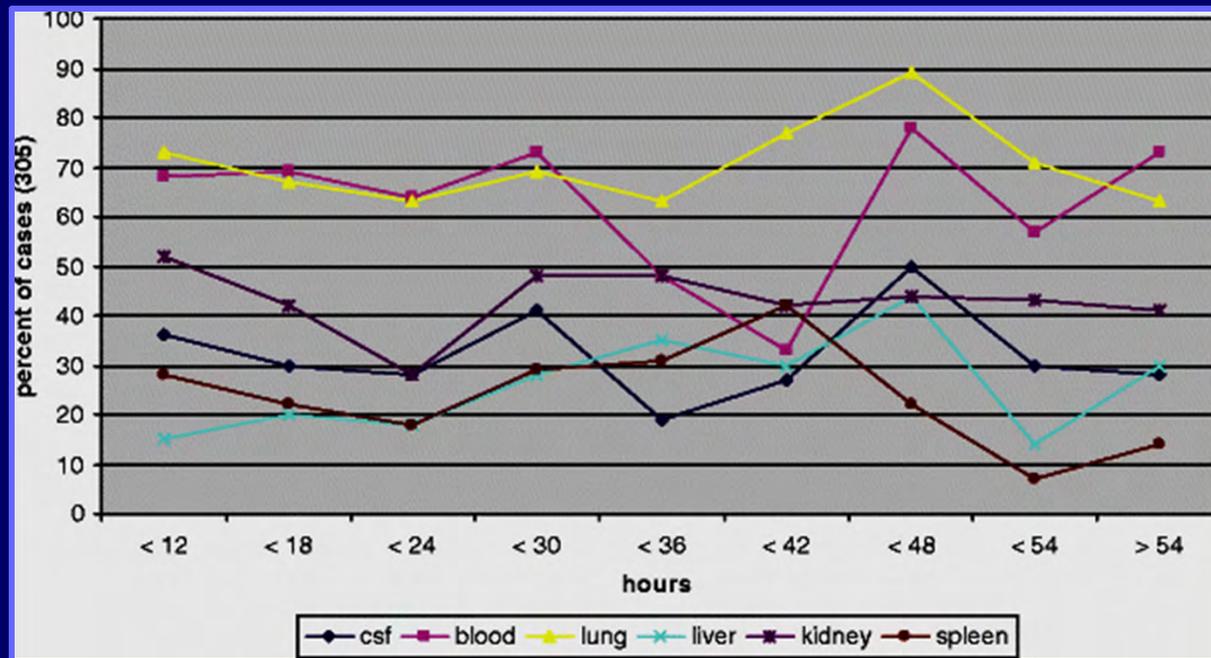
Accuracy not equal to culture of lung aspirates or surgical biopsies obtained during life.



Correlation of tissue sections and culture results ?

Significance of time lapse after death

“historic” studies reported concerns regarding the postmortem interval increasing risk for false-positive cultures (agonal spread, postmortem transmigration)



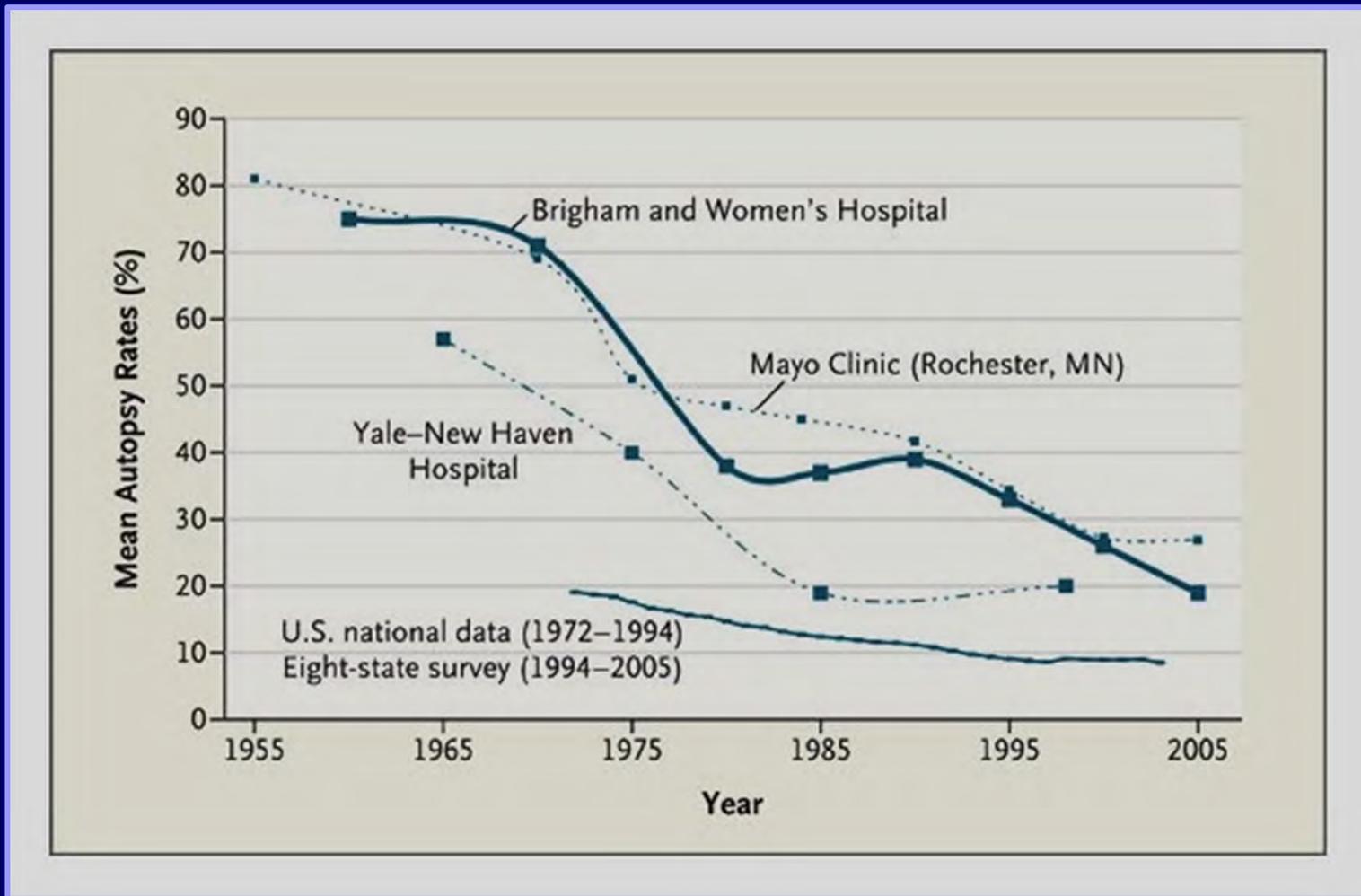
Lobmaier IV, et al. 2009. *Eur J Clin Microbiol Infect Dis*; 28: 1191-1198

Bacterial growth in autopsy specimens according to time after death grouped in 6-h intervals, i.e. < 12 means 6–12 h etc



Postmortem time has NO significant influence on culture results during the first 42 hours after death

Changes in Autopsy Rates over Time



Shojania KG, Burton EC. *N Engl J Med* 2008;358:873-875

Benefits of non-forensic autopsies

Shojania KG, Burton EC. *N Engl J Med* 2008;358:873-875
Burton EC. 2002. *Am J Med Qual*; 17 (2): 56-60

- Education of healthcare practitioners and trainees
- Identification of emerging and/or re-emerging (infectious) diseases
 - HIV/AIDS
 - Legionnaire's disease
 - West Nile Virus
- Quality assurance tool for hospitals
- Contribution to more accurate vital statistics reports
- Identification (or exclusion) of conditions not known during antemortem period, and/or of interest to healthcare providers and family members

Value of Postmortem Cultures

Controversy continues regarding the utility:
Proponents of the usefulness *vs.* those who question utility



Confirmation of antemortem diagnosis



Determine the etiology of an undiagnosed (infectious) disease



Assessment of efficacy of antemortem treatment

Consider the rise in antimicrobial resistance !



Potential for research



Specific utility for forensic autopsy cases

Utility of Postmortem Microbiology Examinations & Cultures

Concerns for routine postmortem microbiology cultures

- Contamination
- Postmortem transmigration (?)

Support

Du Moulin G, et al. 1985. *Hum Pathol*; 16: 539-548

Question utility

Wilson WR, et al. 1972. *Arch Pathol* ; 94: 244-249
Wilson SJ, et al. 1993. *Arch pathol Lab Med*; 117: 986-988



Routine and broad application of postmortem microbiology cultures provide little new information



Lack of studies assessing cost-benefit ratio



Lack of regulatory guidelines (e.g. CAP)



Opportunity to develop more specific guidelines now

Conclusions

- Postmortem cultures are indicated in only a limited number of cases/patients.
- Measures to prevent contamination of cultures must be in place.
- Monomicrobial growth of a common pathogen is indicative of being a true positive culture.
- Polymicrobial growth and isolation of common non-pathogens are likely to represent contaminant organisms.
- The true value of postmortem culture results is determined by correlation of clinical, laboratory, and pathological (autopsy) information.

“Hic locus est ubi mors gaudet succurrere vitae”

(This place is where death rejoices to come to the aid of life)

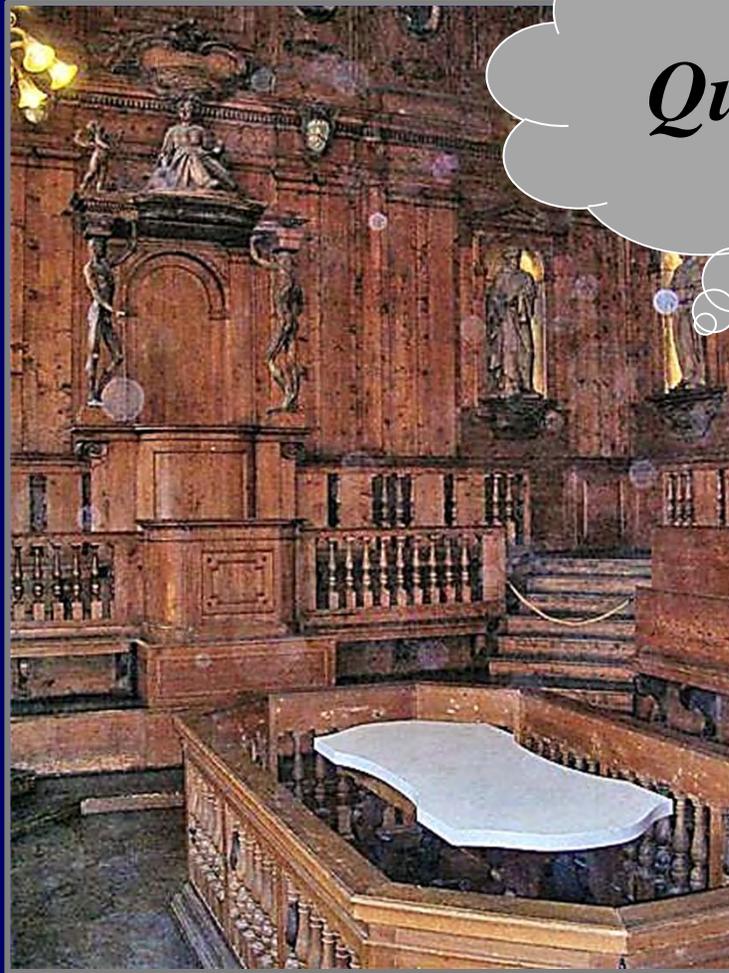


Inscription engraved over the dissection amphitheatre at the University of Bologna, Italy

Everything you ever wanted to know about the postmortem microbiology cultures

- Roberts FJ. 1969. *Canad Med Assoc J*; 100: 70-74
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Thank you for your kind attention!



Questions ?