Biofilm Analysis of MARCoNS Positive Cultures and Its Clinical Implications

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Bacterial Biofilms

- A bacterial biofilm is a structured community of microorganisms encased in an exo-polysaccharide (EPS) which adheres to an inert or living surface.
- Biofilms may be polymicrobial and may consist of not only bacterial cells but also fungi, viruses, proteins, extracellular DNA and other biogenic factors.
- Biofilm bacterial cells are different than free-living growing bacteria, in that they are non-motile and have reduced metabolic activity.
- This reduced activity increases antimicrobial tolerance because many classes of antibiotics are only effective against actively dividing cells by targeting peptidoglycan produced in the cell wall (B-lactams), protein synthesis (Aminoglycosides), or DNA replications (Quinolones).
- Biofilm is a mechanical barrier to antimicrobials and immune system cells, which decreases their effectiveness.
- Biofilm stimulation of the immune system without eradicating the infection causes collateral damage to surrounding tissue and chronic inflammation

Multi-Antibiotic Resistant Coagulase Negative Staphylococcus (MARCoNS) Biofilm Detection

- G.D. Christensen published the first method used to detect biofilm produced by Coagulase Negative Staph (1982; 2).
- Many studies have shown that biofilm producing bacteria including MARCoNS are notoriously difficult to eradicate
- Microbiology Dx has developed a improved biofilm assay method for MARCoNS based upon the original method of Christensen (2)
- The Microbiology Dx modifications yield a semi-quantitative result to better classify the level of biofilm produced.

Multi-Antibiotic Resistant Coagulase Negative Staphylococcus (MARCoNS) Detection

- MARCoNS are of particular concern
- MARCoNS reside in the deep nasal passage, are common in biotoxin illness, and are a marker of low MSH (Melanocyte Stimulating Hormone).
- MARCoNS produce biofilm which form a barrier to immune defenses and anti-infection therapy.
- MARCoNS release exotoxins which lead to increased inflammation and hemolysin which disrupt RBCs and endothelial cells.
- MARCoNS commonly colonize deep nasal passages and can lead to infection.

Multi-Antibiotic Resistant Coagulase Negative Staphylococcus (MARCoNS) Detection

1. The process begins with a nares culture (deep nasal culture) for MARCoNS
2. The nares swab is planted on the appropriate agar media and incubate at 37 degrees C for 24 to 48 hours depending on the growth characteristics of the organism.
3. After incubation, colonies that are characteristic of Coagulase Negative Staphylococcus (CNS) are tested for identification.
4. Once identified as CNS, a susceptibility test is set up and interpreted 24 hours later.
5. If the susceptibility test shows 2 or more resistant or intermediate antibiotics, then the CNS is characterized as MARCoNS.
6. The culture plate may now be forwarded to test MARCoNS for biofilm production.

Multi-Antibiotic Resistant Coagulase Negative Staphylococcus (MARCoNS) Biofilm Detection

1. Materials: MARCoNS culture plate, TSB broth, McFarland Standard (MF) of 0.5 to 1.0 for organisms and controls, Crystal Violet stain. positive control is ATCC 35984 Staph epi (strong biofilm producer) and the negative control is ATCC 12228 Staph epi (non biofilm producer).
2. Each patient tube contains TSB and patient MARCoNS in a standardized concentration and each control tube contains TSB and, the standardized control concentration.
3. After 48 hours of incubation at 37 degrees C, the tubes are worked-up for biofilm by Crystal Violet staining and elution
4. Photometric OD ranges have been established based upon the literature references as Strong 3+, Moderate 2+, Week 1+ and Negative for biofilm production.

Multi-Antibiotic Resistant Coagulase Negative Staphylococcus (MARCoNS) Biofilm Detection Method – Comparative study

1. 233 patients in 27 runs were compared between the Micro Dx Method and the Christensen Tube Method.
2. The Micro Dx Method is a Semi-Quantitative Photometric Tube Method and the Christensen Tube Method (CTM) is a visual observation of the tubes, making it difficult to distinguish a weak from a negative result.
3. A comparison to a photometric method showed a false negative rate of 58% by the CTM (3).
4. The Micro Dx vs. the CTM showed excellent agreement on 161 patients who were biofilm positive and a disagreement on 72 patients due to the error in the CTM in interpreting negatives. The Micro Dx Method interpreted these 72 patients as positive by the photometric.
5. The results on the CTM Method showed 31% of the specimens were negative (a false negative) for biofilm, which the Micro Dx Method showed a week positive biofilm which could be significant in a patient's treatment.

Multi-Antibiotic Resistant Coagulase Negative Staphylococcus (MARCoNS) Biofilm Detection - Comparative study

Biofilm Results	# of patients	%
Strong 3+	63	27
Moderate 2+	31	13.3
Weak +1	73	31.3
Negative	66	28.3
Total Biofilm Positive		71.60%
Total Biofilm Negatives		28.30%

Comparison of Micro Dx MARCoNS Biofilm Results to Literature Studies

- Christensen (1982 and 1985) showed 38% biofilm positive Staph epi isolates from blood contaminants and skin isolates of Staph epi and 64% biofilm positive on IV catheter clinical sepsis isolates. Total isolates 143. (TCM and TCP)
- Mathur (2006) showed 57.8% biofilm positives on 152 clinical isolates of Staph spp. by the Tissue Culture Plate (TCP) Semi Quantitative Method. 14.47% were high biofilm producers, 39.4% were moderate and 46% were weak or no film production isolate from biomedical devises.
- Afreenish (2011) showed 63.7% biofilm positive on 110 isolates from urine, urine catheter tips, IV catheter tips, pus, and nasobronchial lavage specimens. The majority of the bacteria were Staph epi, tested by the TCP Method.

Comparison of Micro Dx MARCoNS Biofilm Results to Literature Studies

- Jain (2009) showed that of 100 isolates, 74% were biofilm positive while only 68% colonizing and 32% commensal isolates were biofilm positive. These were all Staph strains isolated from peripheral IVD, venous blood, site of IVD insertion, nasal mucosa and peripheral IV devices in place for more than 48 hours. All tested by the TCP Method.
- Ruzicka (2004) showed the ability of Staph epi to produce biofilm was compared in 147 clinically significant strains isolated from blood cultures (septicemia) and 147 strains isolated from skin. The strains were examined for the presence of ica operone. The ica operone was found in 92 (62.6%) of blood isolates and in 44 (29.9%) of skin isolates. The CTM showed 53.7% and 22.4% biofilm positive for blood and skin respectively.

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Comparison of Micro Dx MARCoNS Biofilm Results to Literature Studies

Summary – These studies show the biofilm positivity of Staph strains on medical devices and clinically significant specimens by the TCP or the CTM Methods. The Microbiology Dx procedure for the MARCoNS positive biofilm positive specimens is consistent with the literature.

Advantages of the Micro Dx Biofilm Method

The Micro Dx Method vs the Christensen Tube Method (CTM)

- The Micro Dx Method is a spectrophotometric method which is totally objective leading to more consistent results.
- In contrast the Christensen Tube Method (CTM) is visual and studies show a poor correlation from person to person on the weak BF and a negative result.
- The Micro Dx Method uses a defined concentration of Staph epi bacteria based on a McFarland Standard of 0.5 to 1.0. The CTM uses an undefined loop of cultured bacteria.
- The Micro Dx Method is a semi-quantitative method whereas the CTM is qualitative.
- The Micro Dx Method uses full 2 day incubation at 37 degrees C, whereas the CTM uses 1-2 day incubation. Micro Dx Method is more sensitive with the full 2 day incubation.
- The Micro Dx Method can distinguish between weak BF+ and negative biofilm specimens, the CTM cannot.

Advantages of the Micro Dx Biofilm Method

Microbiology Dx vs. TCP Reference Method

- The Micro Dx Method is calculated to be 25 times more sensitive than the TCP Method.
- The Micro Dx Method permits running the biofilm test as the specimens come into the lab, whereas the TCP requires the accumulation of specimens to run in a 96 well format. Therefore, TAT is much faster in the Micro Dx Method.
- The Micro Dx Method performs the culturing as well as the biofilm assay. The sample integrity is much better maintained. Other labs have to send specimens out to another lab.
- Summary – The Micro Dx Method compares favorably to the TCP Reference Method, is more convenient and is more sensitive in its detection ability.

Additional Studies of MARCoNS BF+

Staph aureus vs. MARCoNS and Coag Neg Staph

- A standardized culture for each bacteria was prepared.
- When Staph aureus was combined with either MARCoNS, or Coag Negative Staph on a blood agar plate and incubated at 37 degrees C for 24 hours Staph aureus inhibited the growth of both MARCoNS and Coag Negative Staph.
- This could have significant implications regarding the Nares Microbiome.

Additional Studies of MARCoNS BF+

Multifunctional Studies

- 24 patients who were MARCoNS positive and biofilm positive (18 patients), were tested for susceptibility to EDTA (1%) and were compared for the number of resistant drugs vs. biofilm positive.
- 0.1% EDTA eradicated MARCoNS for all 24 subjects.
- There was no correlation between the strength of the biofilm produced and the number of resistant antibiotics. 15 antibiotics were tested in all cases.
- Summary – The EDTA is very effective in eradicating MARCoNS in-vitro. The lack of correlation between antibiotic susceptibility and the strength of the biofilm formation may be related to the different Staph Coag Negative strains. There are 39 known strains.

Additional Studies

Biofilm Evaluation of Coag Neg Staph – Non MARCoNS

N= 17 patients

Biofilm Formation		
non-MARCoNS Coag Negative Staph		
Strong 3+	3 patients	17.6%
Moderate 2+	4 patients	23.5%
Weak 1+	9 patients	52.9%
Negative	1 patient	6.0%

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Report on the Effectiveness of Antimicrobial Medications in Treating Multiple Antibiotic Resistant Coagulase Negative Staphylococcus (MARCoNS) and MRSA

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Report on the Effectiveness of Antimicrobial Medications in Treating Multiple Antibiotic Resistant Coagulase Negative Staphylococcus (MARCoNS) and MRSA

- Chronic Inflammatory Response Syndrome (CIRS)
  - Describes a constellation of symptoms, associated laboratory findings and test results associated with biotoxin exposure in genetically susceptible individuals.
  - Identified by Dr. Ritchie Shoemaker, M.D.
- CIRS
  - MARCoNS have been found to colonize the deep nasal cavity in 80% of individuals with low MSH hormone.
  - The presence of MARCoNS in the nasopharynx impairs the body to re-establish normal levels of MSH.
  - Adequate MSH is required for recovery from biotoxin induced CIRS. MARCoNS may exist in a biofilm, which makes it more difficult to treat.
  - A deep nasal culture is obtained and sent to Microbiology Dx, Bedford, MA. If Coagulase Negative Staphylococci are present and resistant to more than one class of antibiotics, then treatment is required.
  - (Reference: Sonia Rapport, M.D. and Margaret DiTulio, APRN, MS, MDA, excerpts from essays on Dr. Ritchie Shoemaker's protocol)

Report on the Effectiveness of Antimicrobial Medications in Treating Multiple Antibiotic Resistant Coagulase Negative Staphylococcus (MARCoNS) and MRSA

This report shows the in-vitro evaluations of both the BEG Spray and its individual components.

Conclusion:

A. The BEG solution and its components were tested against defined organisms, Staphylococcus epidermidis ATCC 35984, a strong biofilm producer (MARCoNS positive) And MRSA ATCC 1026 to establish the effectiveness of both the BEG and its individual components in solution.

B. The EDTA 1%, Mupirocin 0.2%, Gentamicin 0.025% and the BEG Spray solution kill the MARCoNS at concentrations less than the original preparation. It appears that EDTA, Mupirocin, and Gentamicin individually and combined in the BEG Spray solution Penetrate the biofilm and kill the MARCoNS.

C. Based on these studies, patients with a Nares culture showing MARCoNS positive be very responsive to this BEG Spray.

Report on the Effectiveness of Antimicrobial Medications In Treating Organisms Isolated from a Deep Nasal (Nares) Culture Using the BEG Spray-Solution

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Other organisms have been isolated from Nares culture, which may be implicated in other conditions such as nasal or sinus infections were tested.

Organisms tested:

Gram positive: MARCoNS, MRSA, Enterococcus species, VRE, Beta-Strep Groups A, B and C, Corynebacterium species, Bacillus species, Alpha Strep – BEG and EDTA effective

Gram negative: E. coli, Klebsiella pneumoniae and oxytoca, Pseudomonas aeruginosa, Citrobacter koseri, Acinetobacter, Salmonella and Serratia species – BEG effective

Mold: Aspergillus, Penicillium, Trichophyton, Microsporum, Cladosporium, Chrysosporium and Pullularia species – BEG and EDTA effective

Yeast: Candida albicans and guilliermondii, Cryptococcus albidus and uniguttulatus, Rhodotorula Mucilaginosa – BEG and EDTA effective

The Corynebacterium species, Bacillus species and yeast were tested against 1% EDTA only which indicates sensitivity to the BEG solution.

All organisms were tested against the standard BEG solution and/or EDTA. The gram positive organisms and yeast showed excellent sensitivity to EDTA.

Report on the Effectiveness of Antimicrobial Medications In Treating Organisms Isolated from a Deep Nasal (Nares) Culture Using the BEG Spray-Solution

Conclusion:

- The BEG Spray (0.2%, 1%, 0.025%) in-vitro is effective in eradicating gram positive and gram negative bacteria, yeast and mold.
- The EDTA 1% eradicated the gram positive bacteria, yeast and mold.
- Based upon these in-vitro studies, patients with a Nares Culture showing any of the organisms listed above should be very responsive to the BEG or EDTA for the gram positive organisms and yeasts.

Report on the Effectiveness of Antimicrobial Oils in Eradicating MARCoNS

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Report on the Effectiveness of Antimicrobial Oils in Eradicating MARCoNS

Introduction:

- Three oils were tested against MARCoNS (Multiple Antibiotic Resistant Coagulase Negative Staphylococcus) for their antimicrobial properties.
- Artemisinin, Curcumin and Oregano Oil Liposomal Suspensions were tested by taking 10-15 colonies of MARCoNS and streaking each of three Blood Agar Plates (BAP). A 50µl aliquot of each oil was applied to 4 locations on each plate and incubated for 18 to 24 hours.

Results:

- The areas where the oils were applied showed inhibition of the MARCoNS in both experiments.
- This confirmed the antimicrobial properties of Artemisimin, Curcumin and Oregano Liposomal Suspensions.

Report on the Effectiveness of Antimicrobial Oils in Eradicating MARCoNS

Conclusion:

- The three oils; Artemisinin, Curcumin and Oregano Oil Liposomal Suspensions showed antibiotic susceptibility to Staph epidermidis ATCC35984, MARCoNS positive and a strong biofilm producer.
- the formulations of the oils are the property of Hopkinton Drug.

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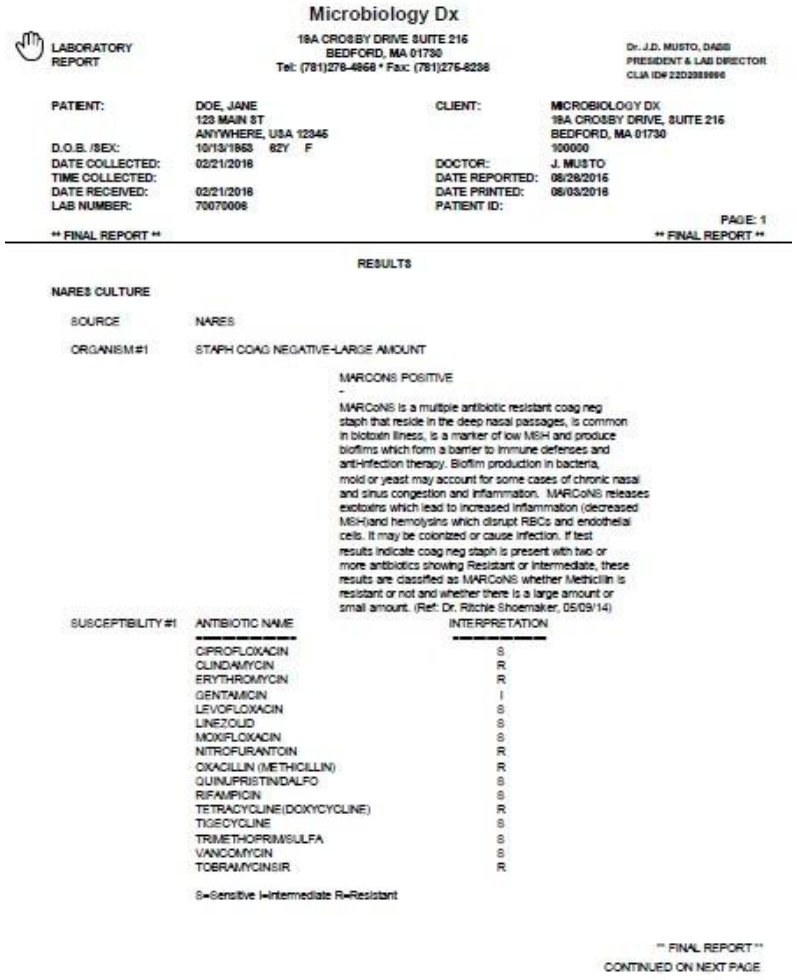
Sample Report from Microbiology DX



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LABORATORY REPORT

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D.O.B. /SEX:	10/13/1953 62Y F		
DATE COLLECTED:	02/21/2018	DOCTOR:	J. MUSTO
TIME COLLECTED:		DATE REPORTED:	06/26/2018
DATE RECEIVED:	02/21/2018	DATE PRINTED:	06/03/2018
LAB NUMBER:	70070006	PATIENT ID:	

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RESULTS

NARES CULTURE

SOURCE NARES

ORGANISM #1 STAPH COAG NEGATIVE-LARGE AMOUNT

MARCONS POSITIVE

-

MARCoNS is a multiple antibiotic resistant coag neg staph that reside in the deep nasal passages, is common in biotoxin illness, is a marker of low MSH and produce biofilms which form a barrier to immune defenses and anti-infection therapy. Biofilm production in bacteria, mold or yeast may account for some cases of chronic nasal and sinus congestion and inflammation. MARCoNS releases exotoxins which lead to increased inflammation (decreased MSH)and hemolysins which disrupt RBCs and endothelial cells. It may be colonized or cause infection. If test results indicate coag neg staph is present with two or more antibiotics showing Resistant or Intermediate, these results are classified as MARCoNS whether Methicillin is resistant or not and whether there is a large amount or small amount. (Ref: Dr. Ritchie Shoemaker, 05/09/14)

SUSCEPTIBILITY #1

ANTIBIOTIC NAME	INTERPRETATION
CIPROFLOXACIN	S
CLINDAMYCIN	R
ERYTHROMYCIN	R
GENTAMICIN	I
LEVOFLOXACIN	S
LINEZOLID	S
MOXIFLOXACIN	S
NITROFURANTOIN	R
OXACILLIN (METHICILLIN)	R
QUINUPRISTIN/DALFO	S
RIFAMPICIN	S
TETRACYCLINE(DOXYCYCLINE)	R
TIGECYCLINE	S
TRIMETHOPRIM/SULFA	S
VANCOMYCIN	S
TOBRAMYCINSIR	R

S=Sensitive I=Intermediate R=Resistant

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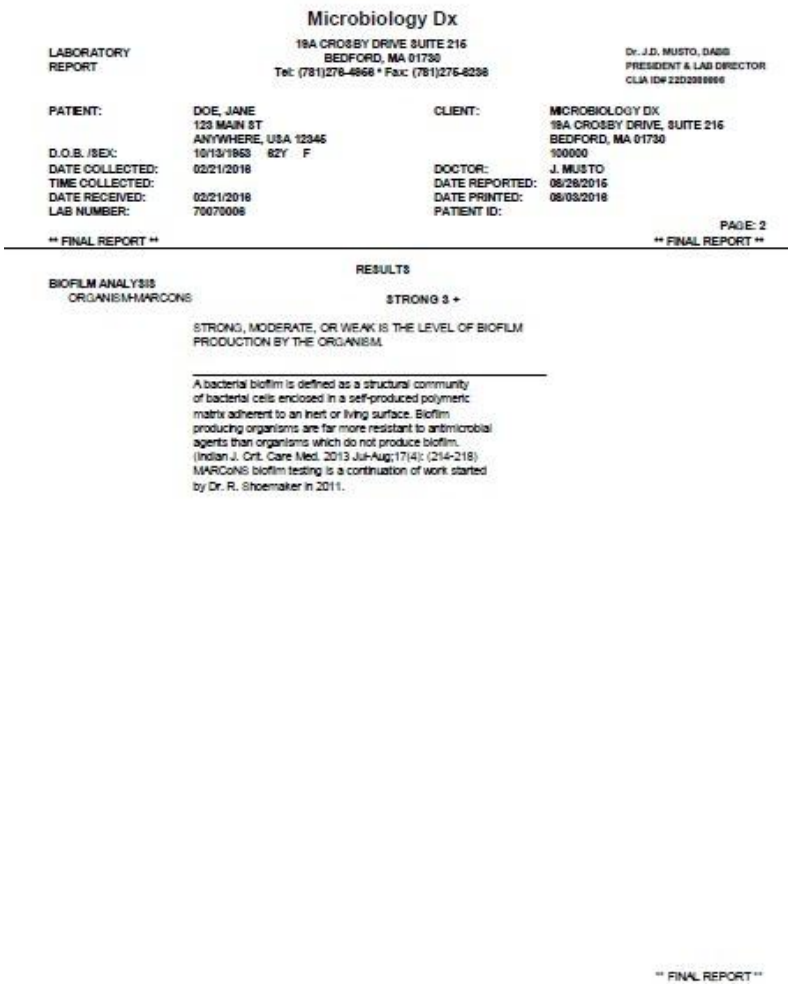

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RESULTS

BIOFILM ANALYSIS

ORGANISM-MARCONS STRONG 3 +

STRONG, MODERATE, OR WEAK IS THE LEVEL OF BIOFILM PRODUCTION BY THE ORGANISM.

A bacterial biofilm is defined as a structural community of bacterial cells enclosed in a self-produced polymeric matrix adherent to an inert or living surface. Biofilm producing organisms are far more resistant to antimicrobial agents than organisms which do not produce biofilm. (Indian J. Crit. Care Med. 2013 Jul-Aug;17(4): (214-218) MARCoNS biofilm testing is a continuation of work started by Dr. R. Shoemaker in 2011.

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Overall Results:

1. The MDx method for MARCoNS biofilm analysis compares favorably to the reference method, is more convenient and more sensitive in its detection ability.

2. Data from several studies show:
   a. Staph aureus coag positive inhibits the growth of Staph coag negative.
   b. EDTA was effective in eradicating MARCoNS in patient specimens and there was no correlation between antibiotic susceptibility and biofilm strength.
   c. Staph coag negative – non MARCoNS were tested and showed a high percentage of biofilm production.

3. Three studies were conducted with Hopkinton Drug and showed the effectiveness of BEG and/or EDTA against MARCoNS, MRSA, other bacteria, mold and yeast. Antimicrobial oils showed effectiveness against MARCoNS.

Discussion:

The biofilm analysis of a strong biofilm producing MARCoNS may explain some of the therapeutic failures. A consideration of the different strains (39) of Staph coag negative before and after treatment may be explained by understanding the effect of biofilms.

Conclusion:

Biofilm of MARCoNS may provide information for enhanced patient treatment.

Learning Objectives:

Understand the clinical implications of biofilm formation for MARCoNS.

Understand the importance of the many different strains of Staph coag negative, the interpretation of the lab report and their effect on patient treatment.

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Thank You !

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