LABORATORY AND CLINICAL DIAGNOSIS OF NOCARDIA

BARBARA A. BROWN-ELLIOTT, MS, MT(ASCP)SM
ASSOCIATE PROFESSOR, MICROBIOLOGY
SUPERVISOR, MYCOBACTERIA/NOCARDIA LABORATORY
UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT TYLER
CONFLICT OF INTEREST

Our lab received funding:

• Merck
• Biomerieux
AMERICAN SOCIETY FOR MICROBIOLOGY
DISTINGUISHED LECTURER PROGRAM

ADVANCING SCIENTIFIC EXCELLENCE ➔ ON THE LOCAL LEVEL

• 55 + years…600 + lectures
• Provided by ASM for ASM Branches
• Funded by ASM & the Waksman Foundation for Microbiology
ASM membership provides access to:

• Cutting-edge science
• Discounted meeting registrations & other products
• Online career resources
• Member-only directory
• Much more!

JOIN OR RENEW TODAY

Visit asm.org/join
Why Join ASM?

• Develop Your Career:
  • ASM Career Resources Website: www.asm.org/careers
  • ASM Online Job Board: asmcareerconnections.org/
• Publish Your Research
• Apply for travel grants and other funding
• Obtain discounts for meetings, conferences and books
• Access ASM member-only listserv discussions & alert lists
• Engage in volunteer leadership opportunities
• Advocate to support the science
• And more…

For complete review of member benefits & member categories visit:
asm.org/join
NOCARDIA

• Aerobic Actinomycete

• Family Nocardiacae

• 1990 only a few species
  • Biochemical assimilation/utilization

• Currently >100 recognized species
  • Gene sequence

• Approximately ½ are human pathogens

NOCARDIA

• Ubiquitous in environment
  • \( H_2O, \) soil, dust, decaying vegetation, etc.
• Some species related to geographical area/climate
  • *N. brasiliensis* – tropical/subtropical (SW, SE, USA)
• Nocardia infections more prevalent in warm, arid climates, dry, dusty, dispersal in wind
NOCARDIA

Morphological / Physiological Characteristics

• Tuberculostearic acids
• Short Chain (40-60 C) mycolics
• Characteristic branching
• Peptidoglycan cell wall; mesodiaminopimelic acid, arabinose, galactose

• CONVENTIONAL ID
• First indication by Gram-stain
  • Branching, filamentous
  • gram + rods
• Often in PMNS, leukocytes, macrophages, mononuclear cells.
NOCARDIA

- In culture – Colonial growth (agar) may appear bacillary or coco-bacillary
- Liquid media – may be more typical filamentous forms
CONVENTIONAL ID OF NOCARDIA

Macroscopic Exam

• Direct: Granules sometimes present
• Colonial: Aerial hyphae; Variable morphology:
  - Chalky (*N. brasiliensis*)
  - Smooth (*N. farcinica*)
• Growth 48-72 hours
• Growth may be inhibited by other bacteria (especially Gram negative rods)
• Hold up to 6 weeks before finalize

N. cyriacigeorgica
Nocardia brasiliensis (by susceptibility pattern)
Nocardia farcinica / Nocardia kroppenstedtii
Nocardia wallacei
NOCARDIA

• Positive Modified AFB Smear (1% H$_2$SO$_4$)

• If ambiguous, transfer to lipid rich media (7H11, LJ) repeat stain after grows

• Include +/- controls

• Often aerial hyphae present

• Few strains little/no hyphae

• Results relate to type of media, age of culture, temp.

• AFB decontamination may ↓ yield

• Examine daily at least first week
CONVENTIONAL ID OF NOCARDIA HPLC

• Separation of *Nocardia* from other aerobic actinomycetes/mycobacteria
  • Mycolic acid peaks
  • Retention time
• Most species of *Nocardia* not differentiated by HPLC alone
Aerobic Actinomycetes
Mycolic Acid Ester HPLC Patterns
**NOCARDIA TAXONOMY**

• Complicated history

• 1888 veterinarian Edmond Nocard isolated gram + org. thought to be cause of bovine farcy (lymphadenitis)

• 1889 named *Nocardia farcinica* by Trevisan

NOCARDIA TAXONOMY (CONT'D)

- Strains deposited in 2 culture collections
  - ATCC 3318 and NCTC 4524
- 1896 Eppinger isolated branching filamentous organism *Cladothrix asteroides* from human brain abscessus subsequently became *Nocardia asteroides*. ("Pseudotuberculosis")

• Strains deposited in 2 collections evaluated: NCTC 4524 was *Mycobacterium* and ATCC 3318 was *Nocardia*

• Additional species named: *N. brasiliensis*, *N. otitidiscaviarum*, *N. transvalensis*

• 1947 First successful treatment with sulfonamides

• 1954 *N. farcinica* ATCC 3318, NCTC 4524 designated type strain
• 1962 Ruth Gordon & colleagues determined 3318 was same as isolate from Eppinger (i.e., *N. asteroides*)

• Appeal to Judicial Commission to change type species to *N. asteroides* (strain ATCC 19247)

• 1970s Uncertain taxonomy

• 1980s Heterogeneity of “*N. asteroides*” complex

• 2000 Recognition of multiple complexes

MOLECULAR ID OF NOCARDIA
16S rRNA GENE SEQUENCE ANALYSIS

• ID by comparison of sequences of unknown isolate to Sequence database

• Commercial sequence MicroSeq 500 (Applied Biosystems) for first 500 bp of 16S rRNA gene
# 10 SPECIES/COMPLEXES FREQUENTLY CAUSE HUMAN DISEASE

<table>
<thead>
<tr>
<th>Species/Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N. abscessus complex</strong></td>
</tr>
<tr>
<td><strong>N. beijingensis</strong></td>
</tr>
<tr>
<td><strong>N. brasiliensis</strong></td>
</tr>
<tr>
<td><strong>N. cyriacigeorgica</strong></td>
</tr>
<tr>
<td><strong>N. farcinica</strong></td>
</tr>
<tr>
<td><strong>N. nova complex</strong></td>
</tr>
<tr>
<td><strong>N. otitidiscaviarum</strong></td>
</tr>
<tr>
<td><strong>N. pseudobrasiliensis</strong></td>
</tr>
<tr>
<td><strong>N. transvalensis complex</strong></td>
</tr>
<tr>
<td><strong>N. wallacei</strong></td>
</tr>
</tbody>
</table>
Identification of Nocardioid species by Partial Sequencing of the 16S rRNA gene: The Sun Sets on Nocardioid asteroids

by Alan McNabb1, Glennia Geddes1, Carol Shaw2, Sultana Mithani3, and Judith Isaac-Renton1,4

Molecular Services Laboratory1, General Bacteriology Laboratory1, Laboratory Services2, British Columbia Centre for Disease Control, and Department of Pathology and Laboratory Medicine3, The University of British Columbia, Vancouver, BC

Nocardioid species are opportunistic pathogens that cause a variety of human infections. Recent descriptions of new species from environmental sources and human infections make maintaining a comprehensive biochemical test panel database difficult and 16S rRNA gene sequencing has been proposed as an alternate means of identification. We acquired 16S rRNA gene sequences for 49 valid and six putative Nocardioid species and 23 taxonomic groups from GenBank and used these sequences to identify 100 clinical and 55 reference Nocardioid isolates. Our database identified 98% of the clinical Nocardioid isolates with N. farcinica (34 isolates) being the most frequently identified species followed by N. euryiogorgoa (23 isolates) and N. nova (22 isolates) representing 69% of all Nocardioid species sent for identification. Nocardioid species validly described since the year 2000, including six isolates of N. beijerinckii, which is the first report of this species from human clinical material, comprised 42% of all isolates indicating the necessity for a current and comprehensive database. Of 54 isolates submitted to us as Nocardioid asteroids or N. asteroides complex, only single isolates were identified as Nocardioid asteroids, suggesting that the previously most commonly reported Nocardioid species is actually a rare cause of human infection.

Keywords: 16S ribosomal RNA, Nocardioidaceae, Nocardioid infections, Molecular diagnostic technique

Résumé

Les espèces Nocardioid sont associées à des agents pathogènes opportunistes responsables d'une variété d'infections chez l'homme. Des descriptions récentes de nouvelles espèces provenant de sources environnementales et d'infections humaines compliquent la tâche de quelques bases de données détaillées d'un panel d'examens biochimiques et de séquençage des gènes ARN 16S a été proposé comme moyen d'identification alternatif. Nous avons obtenu des séquences de gènes ARN 16S pour 49 espèces valides et six espèces reconnues de Nocardioid ainsi que 23 groupes taxonomiques provenant de la base de données GenBank et nous avons utilisé ces séquences pour identifier 100 isolats cliniques et 55 isolats de référence Nocardioid. Notre base de données a identifié 98% des isolats, cliniques de Nocardioid, N. farcinica (34 isolats) l'espèce la plus fréquemment identifiée, suivie de N. euryiogorgoa (23 isolats) et N. nova (22 isolats) représentant 69% de toutes les espèces Nocardioid soumises pour identification. L'espèce Nocardioid, décrite valablement depuis l'année 2000, y compris six isolats de N. beijerinckii, est le premier rapport de cette espèce provenant de matériel clinique humain, comprenant 42% de tous les isolats, indiquant la nécessité d'une base de données actuelle et détaillée. Des 54 isolats qui nous ont été présentés comme étant des complexes Nocardioid asteroids ou N. asteroides, un seul isolat a été identifié comme étant Nocardioid asteroids, suggérant que l'espèce Nocardioid la plus courante rapportée précédemment est en fait une rare cause d'infection chez l'homme.
NOCARDIA ASTEROIDES

• For years considered most common species
• ID based upon inability to hydrolyze casein, tyrosine, xanthine
• Many species of Nocardia are known to be inert
• Confusion of species/relatedness to infection
• Goodfellow noted “N. asteroides cluster”
• 1988 Wallace ID 78 isolates of “N. asteroides complex” with variable antimicrobial susceptibility
EMERGENCE OF THE NOCARDIA ASTEROIDES COMPLEX

• 6 different “drug pattern types”
• Biochemicals relatively inert – now useless
• *N. asteroides* fell into a miscellaneous group with a unique susceptibility pattern

ADVENT OF MOLECULAR METHODS

• Clear molecular differences among the “N. asteroides complex”

• *hsp65, 16S rRNA* gene sequence showed drug patterns/division into 6 taxa
  • Drug Pattern 1: *N. abscessus*
  • Drug Pattern 2: *N. brevicatena/paucivorans*
  • Drug Pattern 3: *N. nova* complex

ADVENT OF MOLECULAR METHODS
(CONT’D)

• Drug Pattern 4: *N. transvalensis* complex
• Drug Pattern 5: *N. farcinica*
• Drug Pattern 6: *N. cyriacigeorgica*

• Drug Pattern 6 (*N. cyriacigeorgica*) was most common and probably responsible for most infections NOT *N. asteroides*

ID OF NOCARDIA SPECIES

• Species level most optimal by gene sequence – most 16S rRNA

• Variable region near 5’ terminus allows ID of majority species by 500 bp sequence

• Some nearly identical in 500 bp – may require full 16S
  • N. abscessus / asiatica / arthriditis
  • N. elegans / veterana
  • N. higoensis / shimofusensis
  • N. farcinica / kroppenstedti
  • N. brasiliensis / vulneris

WHEN TO CALL IT A SPECIES OR A COMPLEX?

Species – Gene sequencing unambiguously identifies an isolate as a distinct species

Complex – Gene sequencing and/or other testing (PRA/susceptibility) indicates it is closely related, or has similar characteristics to a previously identified complex.

Important to communicate what species are included in a complex.
16S rRNA GENE SEQUENCE OF NOCARDIA

• Major problem is high level sequence similarity in species known to be different by DNA-DNA hybridization
  • *N. brevicatena / paucivorans* (99.5% 1,352 bp)
  • *N. kruczakiae / veterana* (99.8% 1,379 bp)

• Must evaluate for multiple copies of 16S with dissimilar nucleotide sequences
  • Overlapping peaks
  • Not resolved by repeat extraction/sequencing
  • Incomplete or incorrect databases
MALDI-TOF

• Variation in protein spectral profiles

• Extraction method

• Database – limited commercial database

• Overall successful ID to complex, some species
  • *N. brasiliensis*, *N. cyriacigeorgica*, *N. farcinica*
  • *N. nova* complex, *N. otitidiscaviarum*

• Problematic

• *N. transvalensis* complex, *N. pseudobrasiliensis*

• *N. abscessus* complex, *N. beijingensis*

• Uncommon species, (e.g., *N. aobensis*, *N. mikamii*, *N. testacea*, etc.)

MULTILOCUS SEQUENCE ANALYSIS (MLSA)

- Concatenated sequences of 4-5 housekeeping genes
  - 16S rRNA, gyrB, secA, hsp65, rpoβ
- Cumbersome and expensive
## Commonly Designated Nocardia Complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Basis for Complex</th>
<th>Species Included in the Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. abscessus</td>
<td>MALDI</td>
<td><em>N. abscessus, N. arthriditis, N. asiatica, N. beijingensis, N. pneumoniae</em></td>
</tr>
<tr>
<td></td>
<td>500 bp 16S</td>
<td>Same without <em>N. pneumoniae</em></td>
</tr>
<tr>
<td>N. nova</td>
<td>MALDI</td>
<td><em>N. nova, N. africana, N. aobensis, N. elegans, N. kruczakiae, N. veterana</em></td>
</tr>
<tr>
<td></td>
<td>16S</td>
<td><em>N. nova, N. africana, N. aobensis, N. elegans, N. kruczakiae, N. veterana, N. cerradoensis, N. mikamii</em></td>
</tr>
<tr>
<td></td>
<td>AST</td>
<td><em>N. nova, N. africana, N. aobensis, N. elegans, N. kruczakiae, N. veterana</em></td>
</tr>
<tr>
<td>Complex</td>
<td>Basis for Complex</td>
<td>Species Included in the Complex</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td><em>N. transvalensis</em> complex</td>
<td>MALDI</td>
<td><em>N. transvalensis, N. wallacei, N. blacklockiae</em></td>
</tr>
<tr>
<td></td>
<td>16S</td>
<td><em>N. transvalensis, N. wallacei, N. blacklockiae</em></td>
</tr>
<tr>
<td></td>
<td>AST</td>
<td><em>N. transvalensis, N. wallacei, N. blacklockiae</em></td>
</tr>
<tr>
<td><em>N. brevicatena/paucivorans complex</em></td>
<td>MALDI</td>
<td><em>N. brevicatena, N. paucivorans</em></td>
</tr>
<tr>
<td></td>
<td>16S</td>
<td><em>N. brevicatena, N. paucivorans</em></td>
</tr>
<tr>
<td></td>
<td>AST</td>
<td><em>N. brevicatena, N. paucivorans</em></td>
</tr>
<tr>
<td><em>N. farcinica</em> complex</td>
<td>16S</td>
<td><em>N. farcinica, N. kroppenstedtii</em></td>
</tr>
</tbody>
</table>
# SUSCEPTIBILITY PATTERNS OF NOCARDIA

<table>
<thead>
<tr>
<th>Complex</th>
<th>Basis for Complex</th>
<th>Species Included in the Complex</th>
</tr>
</thead>
</table>
| Type I  | *N. abscessus* cx| $S = \text{AMP, AMOX/CLAV, CRO, LZD, AMK}$  
$R = \text{CIP, CLAR, IPM}$ |
| Type II | *N. brevicatena/paucivorans* cx | Same as Type I but $S$ to CIP  
$R = \text{GM, CLAR}$ |
| Type III| *N. nova* cx      | $S = \text{AMP, CLAR, LZD, CRO, IPM, AMK}$  
$R = \text{AMOX/CLAV}$ |
| Type IV | *N. transvalensis* cx | $S = \text{CIP, CRO, LZD, IPM}$  
$R = \text{CLAR, ALL AMG}$ |
<table>
<thead>
<tr>
<th>Complex</th>
<th>Basis for Complex</th>
<th>Species Included in the Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type V</td>
<td><em>N. farcinica</em></td>
<td>$S = \text{CIP, LZD, V-IPM}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$R = \text{CRO, CLAR, ALL AMG EXCEPT AMK}$</td>
</tr>
<tr>
<td>Type VI</td>
<td><em>N. cyriacigeorgica</em></td>
<td>$S = \text{CRO, AMK, LZD, IPM}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$R = \text{AMOX.CLAV, CLAR, CIP}$</td>
</tr>
<tr>
<td>Type VII</td>
<td><em>(N. asteroides ATCC 19247)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>N. brasiliensis</em></td>
<td>$S = \text{AMOX/CLAV, DOX/MIN, LZD}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$R = \text{CIP, CLAR, IPM}$</td>
</tr>
<tr>
<td></td>
<td><em>N. pseudobrasiliensis</em></td>
<td>$S = \text{CIP, CLAR, LZD}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$R = \text{AMOX/CLAV, DOX/MIN}$</td>
</tr>
<tr>
<td></td>
<td><em>N. otitidiscaviarum</em></td>
<td>$S = \text{AMK, CIP, LZD}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$R = \text{CRO, AMOX/CLAV, IPM}$</td>
</tr>
</tbody>
</table>
CLINICAL DISEASE ASSOCIATED WITH NOCARDIA

- Ubiquitous in environment
  - Lab Contamination
    - Colonization
    - Non-invasive / saprophytes
- Signs/Symptoms important
- Clinical setting (steroids, transplant, etc.)
- Detection in normally sterile sites
- Large quantities
- Multiple (+) samples

CLINICAL DISEASE ASSOCIATED WITH NOCARDIA

Broad Spectrum of Disease
Localized
Pulmonary
Meningitis
Disseminated
PULMONARY

• Patients with bronchiectasis – colonization common in MAC, CF

• Inhalation of airborne spores or mycelia

• Underlying chronic lung disease

• Seen in patients with long-term steroids

• Cough predominant, thick sputum

• Infiltrates / nodules/ cavitation
EXTRAPULMONARY

• ½ pulmonary nocardiosis also extrapulmonary involvement

• ~20% patients with disseminated disease – extrapulmonary only
  • Spread hematogenously or from asymptomatic or healed pulmonary site mycelia

• Sepsis unusual

• Disseminated also skin, subcutaneous, CNS
CNS

• Disseminated
• Usually ≥1 brain abscess
• ↑ intracranial pressure, headache, nausea, confusion, seizures
• Meningitis rare
PRIMARY CUTANEOUS

• Usually immunocompetent host
• ~80% are *N. brasiliensis*

• Lymphocutaneous infection, superficial cellulitis, localized abscess (lower extremities in adults)
PRIMARY CUTANEOUS (CONT’D)

• Mycetoma – Late stages chronic, slowly progressive, localized, often painless subcutaneous and bone infection (foot)
  • Walking barefoot – repeated trauma/organism in soil
• Tumors, subcutaneous nodules, destructive granuloma fistulas, pus, granules
LYMPHOCUTANEOUS

• Primary pyodermatous lesion (ulcer + pus)
• Chronic drainage, crusting
• Deeply invasive
• Involves lymphatics – abscesses
• Similar appearance “Sporotrichoid”
SUPERFICIAL CUTANEOUS

• Immunocompetent / minor disease
• 1-3 weeks following local trauma / environmental contamination
• Cellulitis, pain, swelling, erythema
• Non-draining
• May spontaneously resolve
• Bacteremia rare

• Catheter-related – removal of catheter, resolves infection

• Non-catheter related bacteremia – high mortality
TREATMENT OF NOCARDIA

• Sulfonamides (since 1940s)

• For CNS infections - ↑ mortality on sulfa alone

• Combination TMP-SMX most common in US

• Most need 3-drug regimens for serious infection
  • TMP-SMX
  • Amikacin
  • Ceftriaxone or Imipenem

• Linezolid
<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>≤8</td>
<td>—</td>
<td>≥16</td>
</tr>
<tr>
<td>Amoxicillin/Clavulanic</td>
<td>≤8/4</td>
<td>16/8</td>
<td>≥32/16</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤1</td>
<td>2</td>
<td>≥4</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>≤2</td>
<td>4</td>
<td>≥8</td>
</tr>
<tr>
<td>Doxycycline/Minocycline</td>
<td>≤1</td>
<td>2 – 4</td>
<td>≥8</td>
</tr>
<tr>
<td>Linezolid</td>
<td>≤8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>≤1</td>
<td>2</td>
<td>≥4</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤4</td>
<td>8 – 16</td>
<td>≥32</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>≤2</td>
<td>4</td>
<td>≥8</td>
</tr>
<tr>
<td>TMP-SMX</td>
<td>≤2/38</td>
<td>—</td>
<td>≥4/76</td>
</tr>
</tbody>
</table>
• *Nocardia* species described from environmental sources are not uncommon from clinical samples

• Phenotypic methods are not sufficient for the ID of *Nocardia*.

• **Molecular methods** of identification are necessary to ID most species of *Nocardia*.

• Accurate ID of *Nocardia* sp. may require sequencing of genes other than the 16S rRNA gene.
In North America:

• *N. asteroides* is a rare human pathogen (<1% incidence)

• *N. cyriacigeorgica*, *N. farcinica*, and *N. nova* (sensu stricto) are the 3 most commonly isolated human pathogens of *Nocardia*.

• Undescribed species are not uncommon in clinical samples
CAVEATS FOR ID OF NOCARDIA

• Full sequencing of the 16S rRNA, hsp65, sec A 1 or gyr B genes can identify all species of Nocardia if they are sufficiently similar to a type strain.

• Always research unusual species


• LSPN List of Prokaryotic Names with Standing in Nomenclature http://www.bacterio.cict.fr/index.html
CAVEATS FOR ID OF NOCARDIA (CONT’D)

• Has the species been described in the literature? Is it a valid species?
• Has it been implicated as a human pathogen?
• If your laboratory is unable to ID an isolate to species or complex level, consider sending the isolate to a reference laboratory, especially if the isolate is clinically significant.
ALGORITHM FOR ID OF NOCARDIA

Nocardia

Clinical Isolate

MALDI

Extraction/analysis

ID to species/complex

16S rRNA or MLSA

ID to species, complex

Refer to reference lab

ACKNOWLEDGMENTS

Richard J. Wallace, Jr., M.D.
Director, Mycobacteria/Nocardia Lab

Adrian Almodovar
Megan Ashcraft
Linda Bridge
Georgie Bush
Mary Davis
Bibiana Gonzalez-Ramirez
Elena Iakhiaeva

Amber McKinney
Jacob Mooney
Kelly Ritter
Katie Shipp
Terry Smith
Sruthi Vasireddy
Becky Wilson

Joanne Woodring
Thank You!
# American Hemerocallis Society
New Registered Cultivar Data Sheet

<table>
<thead>
<tr>
<th>Date</th>
<th>Seedling Number</th>
<th>Cultivar Name</th>
<th>Resistant</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/30/2018</td>
<td>T62-1</td>
<td>Clyde Elliott</td>
<td>1</td>
<td>38</td>
</tr>
</tbody>
</table>

| Reproductive | | | | | |
|--------------|---|---|---|---|
| Flower       | 7.12 | M | 1 | 3 | 20 | 5.6 | 13.07 |
| Foliage      | 0.5 | 0.5 | 0.5 | 0.5 |

**Parentage:**
(Spacecot Gold Banana × Hnk lemonade Party)

**Description:**
medium pale yellow with slight ruffling, ruffled edge, and a green throat

<table>
<thead>
<tr>
<th>Deposit</th>
<th>Tetraploid</th>
<th>Single</th>
<th>Double</th>
<th>Monocline Percent</th>
<th>Polyploid</th>
<th>Polyploid Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Scale</th>
<th>Petal Width</th>
<th>Petal Width metric</th>
<th>Petal Length</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.000</td>
<td>0.000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
ANY QUESTIONS?