SIMPLIFYING GROUP A STREPTOCCI DETECTION WITH CHROMOGENIC MEDIA

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DISCLOSURES

- I have served as a consultant for Thermo Fisher Scientific and COPAN Diagnostics
- I developed the presentation and the opinions expressed are my own

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BACKGROUND

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STREPTOCCUS PYOGENES BACKGROUND

Incidence

- ➢ United States:
  - o Approximately 10,000,000 Non invasive Group A Strep cases causing acute pharyngitis or impetigo
  - o About 9,000-11,500 cases of invasive GAS disease occur each year in the United States, resulting in 1,000-1,800 deaths annually (3.2-3.9 per 100,000 population)
  - o STSS and necrotizing fasciitis each comprise an average of about 6%-7% of these invasive cases
- ➢ Europe:
  - o 2003-2004: 3/100,000 cases
  - o Skin Infections in 25% of cases
  - o 32% of patients having cellulitis
  - o 8% necrotizing fasciitis
  - o The overall 7-day case fatality rate was 19%;
  - o 44% among patients who developed streptococcal toxic shock syndrome

PHARYNGITIS (TONSILLITIS)

- Specifically refers to inflammation of the pharynx.
- Can be exudative, ulcerative, or membranous
- Uvulitis can occur concomitantly or as an extension of serious systemic disease e.g., epiglottitis.
- Most frequent etiology in patients > 3 is GAS but can also occur at an earlier age.
- Throat (not NP) cultures on selective media, antigen detection, or amplification assays are all appropriate for Dx.
- Streptococcal serologies (ASO, AHT, ADB) are not.

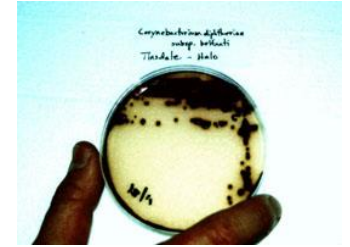MEDICAL COLLEGE OF WISCONSIN
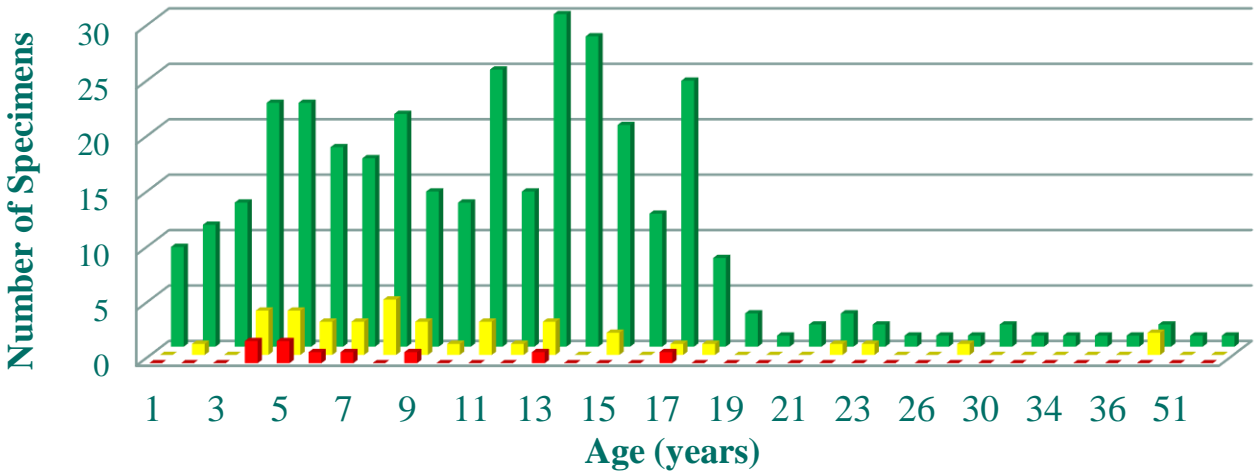
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PHARYNGITIS (TONSILLITIS)

- Suppurative complications of GAS pharyngitis include OM, sinusitis, mastoiditis, peritonsillar abscess, and retropharyngeal abscess.
- Other β-hemolytic streps (B,C,&G) can cause pharyngitis. The latter 2 can be food-born. None of these can lead to significant post-streptococcal disease.
- Culture results will not distinguish carriers from disease although colony quantity usually helps (assuming proper collection).

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PHARYNGITIS (TONSILLITIS)

- Other bacterial etiologies include:

  - *M. pneumoniae*

  - *Arcanobacterium haemolyticum*

  - *Francisella tularensis*

  - *Haemophilus ducreyi*

  - *Neisseria gonorrhoeae*

  - *Corynebacterium diphtheriae* (requires special medium for presumptive identification).

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GAS DISTRIBUTION BY AGE

Anderson *et al*, ID Week, Abstract 189. 2012

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LABORATORY DIAGNOSIS
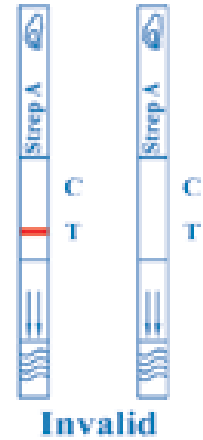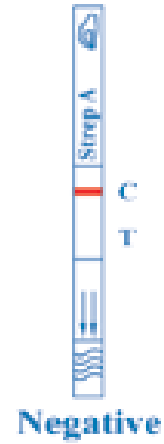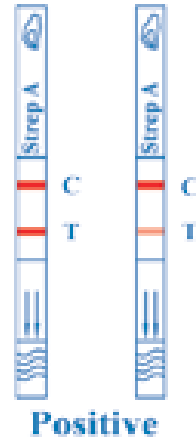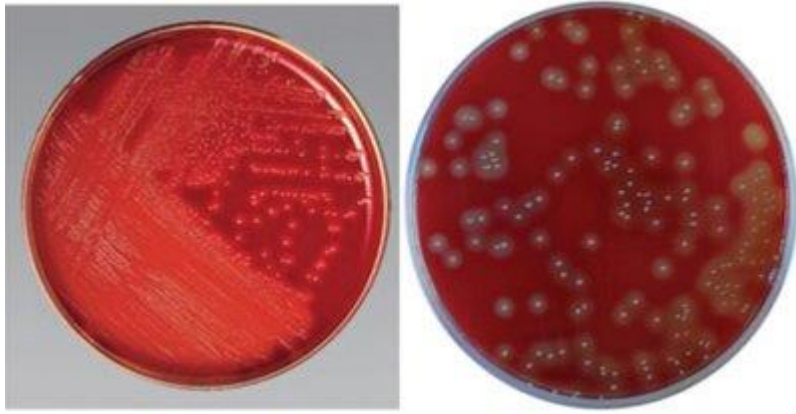
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WHAT DO THE IDSA GUIDELINES TELL US?

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CLINICAL PRACTICE GUIDELINE FOR THE DIAGNOSIS AND MANAGEMENT OF GROUP A STREPTOCOCCAL PHARYNGITIS: 2012 UPDATE BY THE INFECTIOUS DISEASES SOCIETY OF AMERICA

Stanford T. Shulman, Alan L. Bisno, Herbert W. Clegg, Michael A. Gerber, Edward L. Kaplan, Grace Lee, Judith M. Martin, and Chris Van Beneden

HOW SHOULD THE DIAGNOSIS OF GAS PHARYNGITIS BE ESTABLISHED?

- "Swabbing the throat and testing for GAS pharyngitis by rapid antigen detection test (RADT) and/or culture should be performed because the clinical features alone do not reliably discriminate between GAS and viral pharyngitis except when overt viral features like rhinorrhea, cough, oral ulcers, and/or hoarseness are present."
- "In children and adolescents, negative RADT tests should be backed up by a throat culture. Positive RADTs do not necessitate a back-up culture because they are highly specific."
- "Routine use of back-up throat cultures for those with a negative RADT is not necessary for adults in usual circumstances, because of the low incidence of GAS pharyngitis in adults and because the risk of subsequent acute rheumatic fever is generally exceptionally low in adults with acute pharyngitis."
  - "Physicians who wish to ensure they are achieving maximal sensitivity in diagnosis may continue to use conventional throat culture or to back up negative RADTs with a culture."
- "Anti-streptococcal antibody titers are not recommended in the routine diagnosis of acute pharyngitis as they reflect past but not current events"

WHAT CAN WE EXPECT FROM OUTCOMES STUDIES?

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Distribution of GAS-positive results for each of the assays tested.

Group A Streptococcus Test Comparison
384 throat swabs

Culture
4

321
Negative by all methods

0

21

30

1
Directigen™ Rapid Antigen Immunoassay

0

7
LightCycler Strep-A PCR Assay

Uhl J R et al. J. Clin. Microbiol. 2003;41:242-249

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COST, EFFECTIVENESS AND COST-EFFECTIVENESS RATIOS OF BASE CASE ANALYSIS

Strategy	Individual cost (€)	Effectiveness	Cost[a] (€)	Effectiveness[a, b]	C/E
Treat all	55.79	0.899	223,170,684	3,594,546	62.09
Clinical scoring	54.67	0.908	218,672,177	3,632,055	60.21
Rapid test	49.12	0.959	196,467,502	3,836,061	51.22
Culture	86.04	0.974	344,146,464	3,895,304	88.35
Rapid test + culture	74.70	0.957	298,786,412	3,826,582	78.08
Clinical scoring + rapid test	48.78	0.962	195,124,106	3,847,380	50.72

Giraldez-Garcia, C. *et al* European Journal of Pediatrics. 2011 Aug;170(8):1059-67

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Complications of GABHS, treatment unnecessary and adverse reactions to penicillin therapy

Strategy	Complications occurring		Treatment unnecessary	Allergic reactions[b]		Deaths
Treat all	SC	6,000	3,000,000	Severe	20,000 (15,000)	40 (30)
	RF	60		Mild	379,960 (284,970)	
Clinical scoring	SC	7,440	2,670,000	Severe	18,050 (13,350)	36 (27)
	RF	74		Mild	342,914 (253,623)	
Rapid test	SC	8,400	660,000	Severe	7,800 (3,300)	16 (7)
	RF	84		Mild	148,184 (62,693)	
Culture	SC	7,200	30,000	Severe	4,900 (150)	10 (0)
	RF	72		Mild	93,090 (2,850)	
Rapid test + culture	SC	6,120	683,400	Severe	8,392 (3,417)	17 (7)
	RF	61		Mild	159,431 (64,916)	
Clinical scoring + rapid test	SC	9,696	587,400	Severe	7,167 (2,937)	14 (6)
	RF	97		Mild	136,159 (55,597)	

WHAT IMPROVEMENTS HAVE OCCURRED IN DIAGNOSTICS?

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CHROMOGENIC MEDIA

- Appropriately colored colonies are easily recognized by the laboratory professional
- Typically offer high sensitivity and specificity
- Can allow easy identification of low quantities of clinically significant organisms
- Can allow easy separation of normal flora from clinically significant organisms
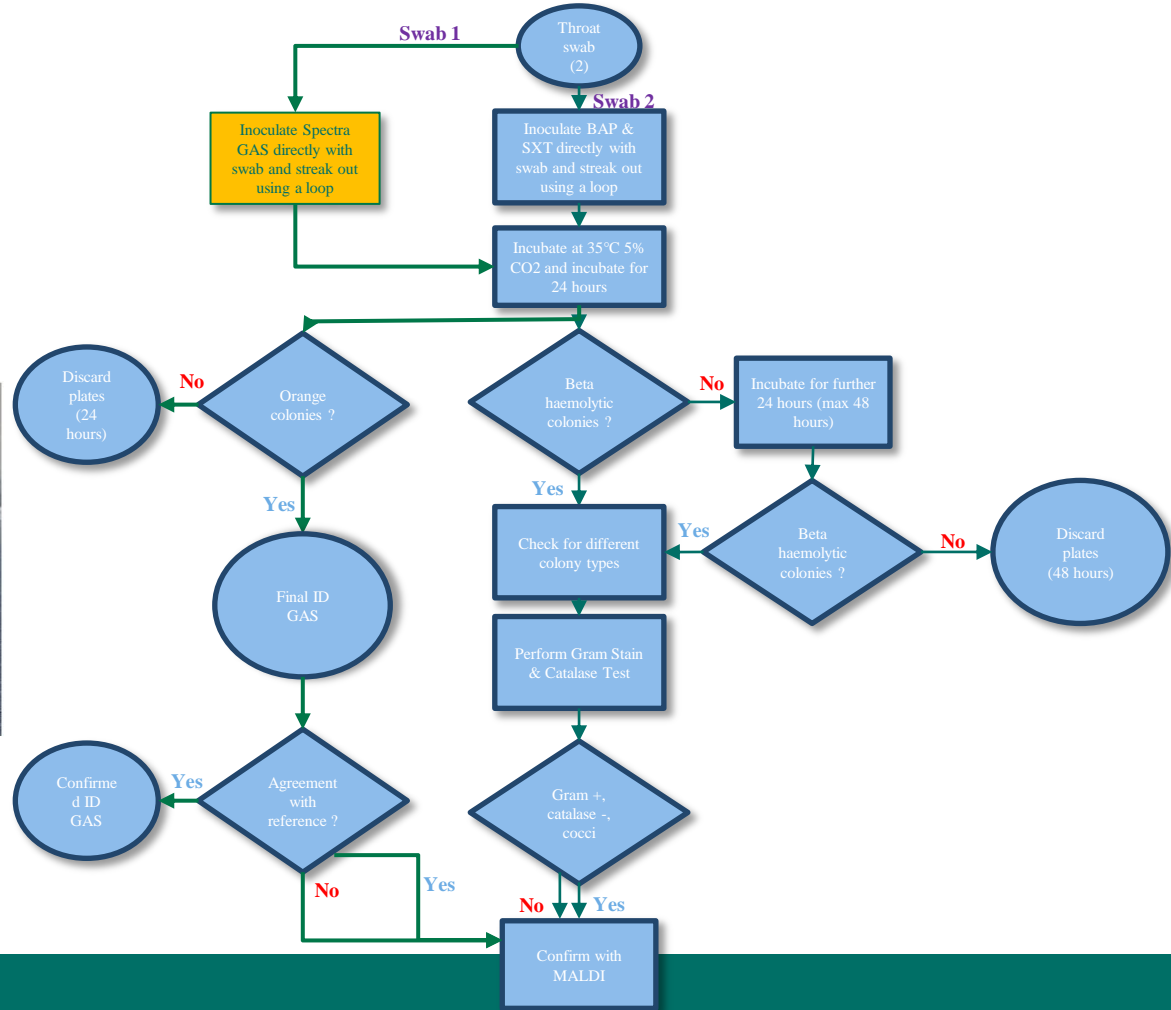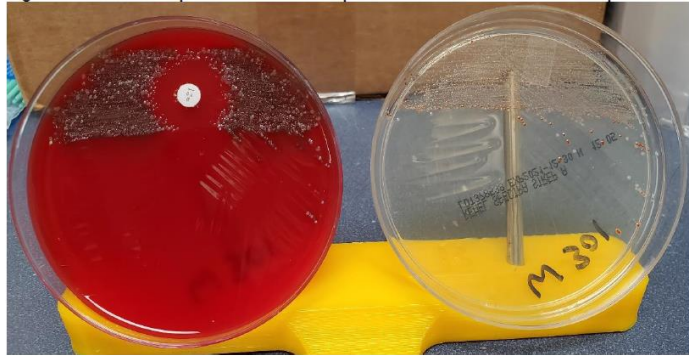
THERMO SCIENTIFIC™ SPECTRA ™ STREP A AGAR

- S. *pyogenes* will appear as orange colonies on the plate
- Non-S. *pyogenes* will be inhibited or appear alternate color
  - *E. coli* – inhibited
  - *S. aureus* – inhibited
  - *S. dysgalactaie* – white colonies
  - *E. faecalis* – blue-green colonies

TYPICAL PATIENT SPECIMEN AND STUDY PROTOCOL

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PERFORMANCE OF CHROMOGENIC MEDIA

Performance	Non-spiked specimens		Specimens spiked with ≥1 x 10^5 CFU/mL of GAS		Overall	
	Spectra GAS Agar	Reference method BAP with bacitracin disc	Spectra GAS Agar	Reference method BAP with bacitracin disc	Spectra GAS Agar	Reference method BAP with bacitracin disc
True positives (TP)	22	17	22	18	44	35
False positives (FP)	1	21	0	1	1	22
True negatives (TN)	343	325	6	6	349	331
False negatives (FN)	0	3	1	4	1	7
Sensitivity	100.0%	85.0%	95.6%	.8%	97.8% (95% CI=96.3-99.2)	83.3% (95% CI=79.7-87.0)
Specificity	99.7%	93.9%	100.0%	85.7%	99.7% (95% CI=99.2-100)	93.8% (95% CI=91.4-96.2)
Positive predictive value	95.7%	44.7%	100.0%	94.7%	97.8% (95% CI=96.3-99.2)	61.4% (95% CI=56.6-66.2)
Negative predictive value	100.0%	99.1%	85.7%	60.0%	99.7% (95% CI=99.2-100)	97.9% (95% CI=96.5-99.3)

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OTHER ADVANCES

- Development of molecular tests for:
    - *S. pyogenes* - IVD
    - *S. pyogenes* + *S. dysgalactaie* – IVD
    - Comprehensive throat culture replacement – in development
- POC molecular tests to replace antigen tests
    - CLIA waived options
    - At home options – in development
- AI-supported reading algorithms

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A DIGITAL TECH LIVING IN A DIGITAL WORLD

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Evolution of automation in the clinical laboratory

- Historical limitations for automation in Microbiology
  - Specimen variety of Microbiology
    - Multiple specimen types – blood, urine, sputum, sterile fluids, devices, etc.
    - Various collection devices
    - Different manipulation needed
  - Complexity of data interpretation
    - Not a measurement of 1 target for detectors
      - pH or leukocyte esterase
      - Each bacteria is its own component
    - Complex rules and non-sterile specimens
  - Cost of automation
    - Volume too low to justify cost

Evolution of automation in the clinical laboratory

Small steps

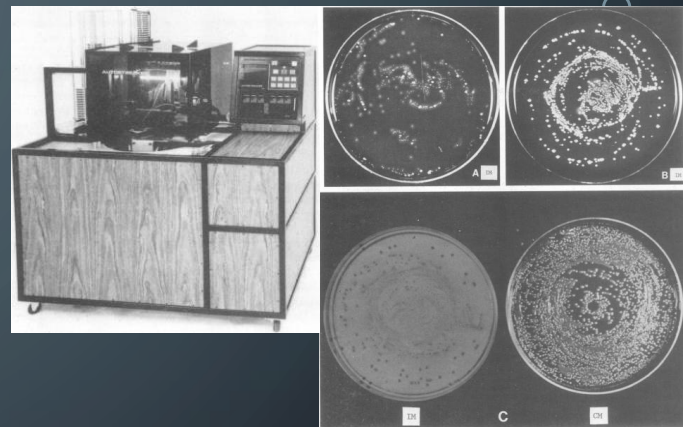- Several advancements in automation
  - Blood culture monitoring
    - Only blind subs after 5 days
  - Bacterial identification
    - API strips to Phoenix™, Vitek™, and Thermo Scientific™ Sensititre™
  - Antimicrobial Susceptibility testing (Phoenix Vitek, Sensititre)
    - Removed the manual inoculation and reading of broth microdilutions
  - Extraction methods for molecular testing
  - Molecular assays

Evolution of Automation in the clinical laboratory

- Changes in the lab
  - Laboratory consolidation
  - Increase in volumes
  - Reduction in trained workforce – 50% fewer between 1983-2008
  - Liquid-based transport – Eswab™

- Implementation of Specimen processing
  - Plate Inoculation
    - Concept - 1978
    - Walk-Away Specimen Processor (WASP®DT)
      - Loop based streaking
    - InoquIA-BD
      - Metal ball and magnets

InoquIA is a trademark of BD Kiestra B.V. private limited liability company

Tilton and Ryan 1978. National Accrediting Agency of Clinical Laboratory Sciences. 2009. Bourbeau and Ledeboer 2013

Total Laboratory Automation

WASPLab®, COPAN Diagnostics

Kiestra™ TLA, BD

How digital image analysis is performed?

- Software analysis*
  - Image differentials

Time = 24 hours
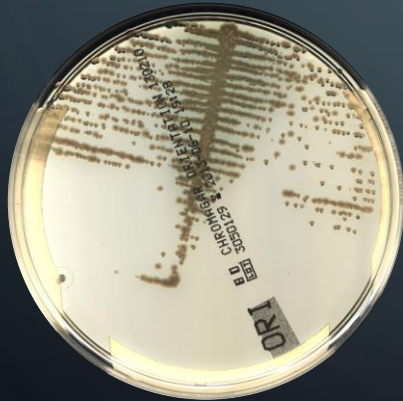
Time = 0 hours

Differential

*Developed by COPAN
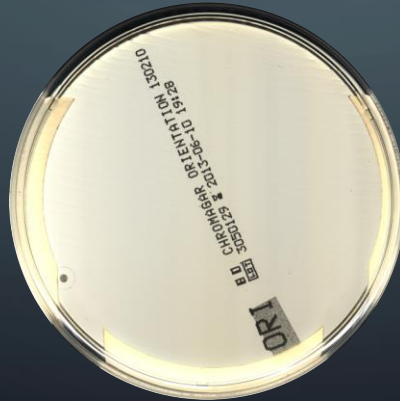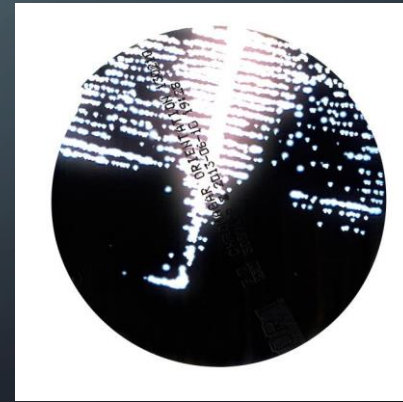
Differentiating negative and "non-negative" chromogenic plates

- Differential analysis*
  - Each pixel
  - Color change

- Develop threshold for agar*
  - Convert RGB to HSV
    - H = Hue
    - S = Saturation
    - V = Value (brightness)

- First studies
  - VRE chromogenic media plates
  - MRSA chromogenic media plates

*Developed by COPAN
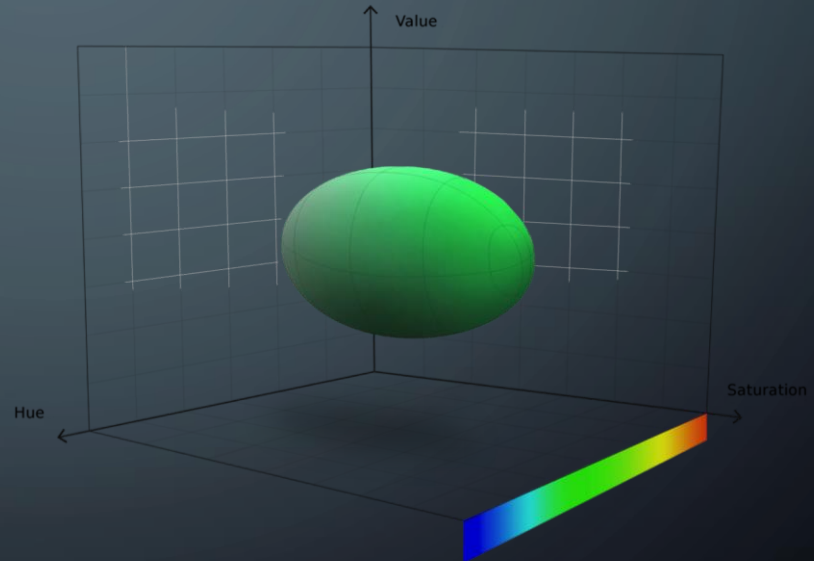
Study design for evaluating chromogenic agar

- 3 – 4 sites per study

- Eswab™ specimens
    - VRE – 104,730 rectal swab specimens
    - MRSA – 57,690 nasal swab specimens

- Media
    - VRE – 2 chromogenic media plates
    - MRSA – 3 chromogenic media plates

- Reference method
    - Manual reading by trained technologists

- Discordant analysis
    - Images reviewed by a supervisor

Manual

Automated

Compare

VRE Results

Performance of WASPLab[TM] digital imaging of VRE plates compared to manual reading										
Clinical test site	No. of specimens tested	Results (no.)[a]				Performance (% [95% CI])[b]		PPV[c] (%)	NPV[c] (%)	Prevalence
		MP/AP	MN/AN	MN/AP	MP/AN	Sensitivity	Specificity			
1	11,438	1,474	9,129	835	0	100 (99-100)	91.6 (91-92)	64	100	12.9%
2	75,518	2,822	64,535	8,161	0	100 (99-100)	88.8 (88-89)	26	100	3.7%
3	17,774	2,107	14,315	1,352	0	100 (99-100)	91.4 (91-92)	61	100	11.8%
Total	104,730	6,403	87,979	10,348	0	100 (99-100)	89.5 (89-90)	38	100	6.1%

[a]MP/AP, manual Pos automation Pos; MN/AN, manual Neg/automation Neg; MN/AP, manual Neg/automation pos; MP/AN, manual Pos/automation Neg.

[b] CI, confidence interval.

[c]PPV, Positive Predictive Value; NPV. Negative Predictive Value

Faron et al. 2016: PMID 27413193

Discrepant categorization

Automation positive 2nd Manual positive
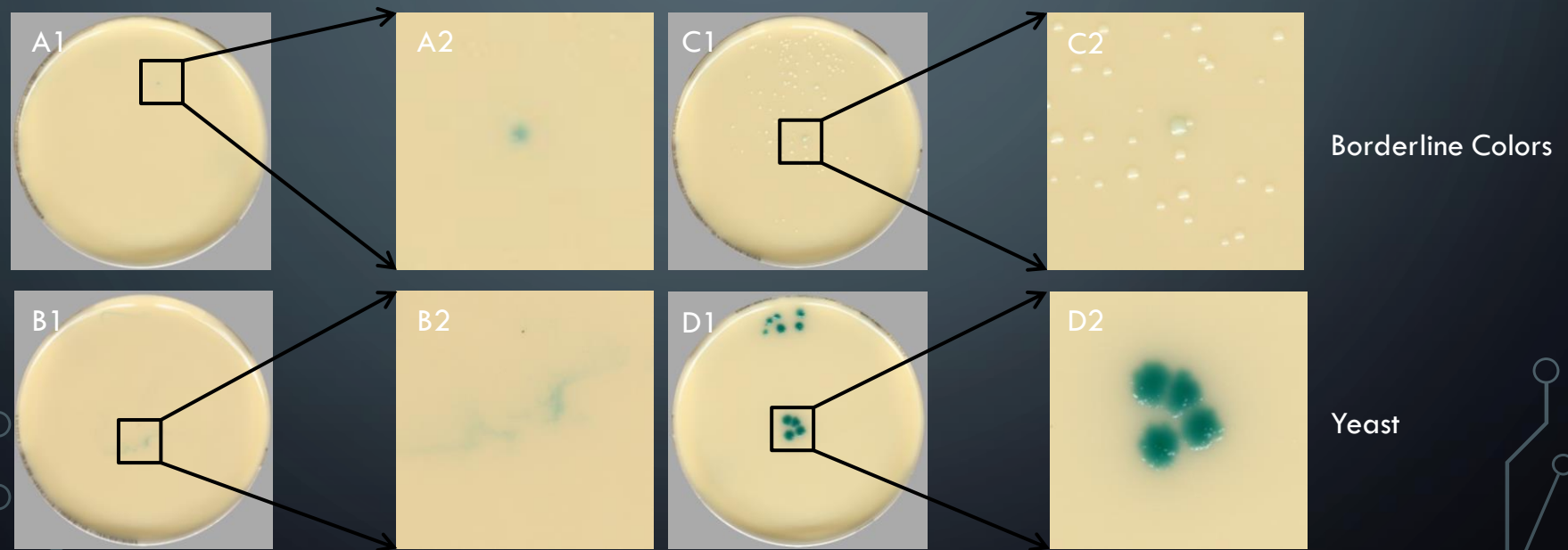
Borderline Colors

Yeast

Residual Matrix

Faron *et al.* 2016: PMID 27413193

Discrepant analysis

Discrepant analysis of Manual Negative/Automation Positive Plates				
Discrepant Category	MN/AP[a]	Automation Positive 2nd Manual Positive	Residual Matrix/Yeast	Borderline Colors
Total number of plates	10,348	499	8,234	1,616
Colorex VRE	8996	432	7684	881
Thermo Scientific™ Oxoid™ VRE	1352	67	550	735
[a] Manual Negative/Automation Positive				

Faron *et al.* 2016: PMID 27413193

MRSA RESULTS

| Performance of WASPLab™ digital imaging compared to manual reading								
Clinical test site	No. of specimens tested	Results (no.)[a]				Performance (% [95% CI])[b]		Prevalence
		MP/AP	MN/AN	MN/AP	MP/AN	Sensitivity	Specificity	
1	5604	119	5266	219	0	100 (96-100)	96.0 (95-96)	2.1%
2	41064	680	36333	4051	0	100 (99-100)	90.0 (89-90)	1.6%
3	2217	162	1898	157	0	100 (97-100)	92.4 (91-93)	7.3%
4	8805	406	7616	783	0	100 (99-100)	90.7 (90-91)	4.6%
Total	57690	1367	51113	5210	0	100 (99-100)	90.7 (90-91)	2.4%

[a]MP/AP, manual Pos automation Pos; MN/AN, manual Neg/automation Neg; MN/AP, manual Neg/automation pos; MP/AN, manual pos/automation Neg.
[b] CI, confidence interval.

Faron et al. 2016: PMID 26719443

MRSA DISCREPANT ANALYSIS

Discrepant analysis of Manual Negative/Automation Positive Plates			
Discrepant Category	MN/AP[a]	Automation Positive 2nd Manual Positive	Residual Matrix	Borderline Colors
Number of plates	5210	153	1189	3868
[a] Manual Negative/Automation Positive			

Faron *et al.* 2016: PMID 26719443

What about GAS?

STUDY – CHLA/UCLA STUDY

- Single center study

- 480 pharyngeal specimens - ESwab
  - Pediatric patients

- SOC- Lyra direct strep PCR
  - Frozen post testing

- Thawed and tested
  - Colorex™ Strep A agar
  - Blood agar plate w/bacitracin disk

- Discordant specimen
  - Second technologist read
  - Chrom negative PCR + - sent to Wisconsin diagnostic labs for Lyra PCR repeat test

- Composite reference gold standard
  - Positive = GAS from any culture media or culture negative but PCR pos 2x
  - MALDI ID confirmation

Colorex is a trademark of Rambach, Alain.

Manual

Automated

Compare

Dein Bard *et al.* 2019: PMID 31434725

GAS Sensitivity and Specificity

Method	Sensitivity (%) (no. positive/total no.)	Specificity (%) (no. positive/total no.)	Positive predictive value (%)	Negative predictive value (%)
Lyra molecular assay	96.9 (93/96)	100 (384/384)	100	99.2
Manual reading of Colorex Strep A agar images	87.5 (84/96)	97.7 (375/384)	90.3	96.9
PhenoMATRIX™ reading of Colorex Strep A agar images	90.6 (87/96)	94.0 (361/384)	79.1	97.6
Manual detection of β-hemolytic colonies on BAP images	83.3 (80/96)	69.3 (224/384)	44.7	93.3
Manual detection of β-hemolytic colonies on BAP images with any zone of inhibition with bacitracin disk	39.5 (38/96)	83.1 (319/384)	36.9	84.6

PhenoMATRIX is a trademark of COPAN ITALIA.

GAS result breakdown

- 480 – 96 defined as true positive

- 3 specimens missed by manual read
  - PCR positive not MALDI confirmed

- PCR picked up 9 missed by all culture

- PCR missed 3 picked up by both manual and automated reads

- PCR repeat was pos on 12/18 tests

Dein Bard *et al.* 2019: PMID 31434725
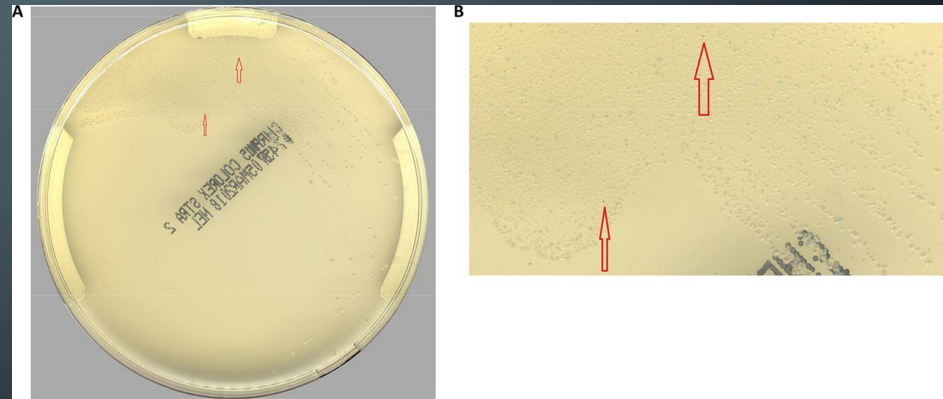
Culture False Positives

- 9 isolates identified with orange colonies
  - Detected both manual and positive

- 14 Automation FP results
  - 6 light brown colonies
  - 8 residual matrix coloration
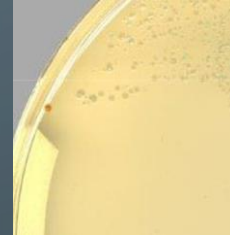
FP culture ID	# cultures
Kocuria rhizophilia	1
S. aureus	1
S. simulan	1
L. rhamnosus	1
R. mucilaginosa	1
S. salivarius	2
S. agalactiae	2

Possible impact

- Culture – previously demonstrated highly effective workflow
  - Chromogenic – more sensitive
  - Automation increased sensitivity

- Culture workflow – expensive
  - Chromogenic agar adds expense
  - Automation can reduce cost
    - < FTE for negatives
      - VRE study ~ $14,280.00, 357 tech hours
      - MRSA study ~ 8,306.00, 208 tech hours

Complications of GABHS, treatment unnecessary and adverse reactions to penicillin therapy

Strategy		Complications occurring	Treatment unnecessary	Allergic reactions[b]		Deaths
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Summary

- Standardization of specimen collection along with improvements to automation is creating an impact in clinical microbiology

- AI for image analysis is highly sensitive and can improve laboratory workflow
  - Similar results in multiple media and targets including: VRE (PMID27413193), MRSA (PMID26719443) GAS (PMID31434725), GBS (PMID33087433), and CPSe agar (PMID31694967)
  - Other targets to come – ex. yeast

- PhenoMATRIX® software advancements
  - Identifying and counting colonies
    - Pos/Neg urine cultures (PMID3941690)
  - Reading zones of inhibition (PMID2833189, 3173376, 35217936)

- Future
  - Differentiating colony morphology
  - Preliminary ID based on morphology

Questions?