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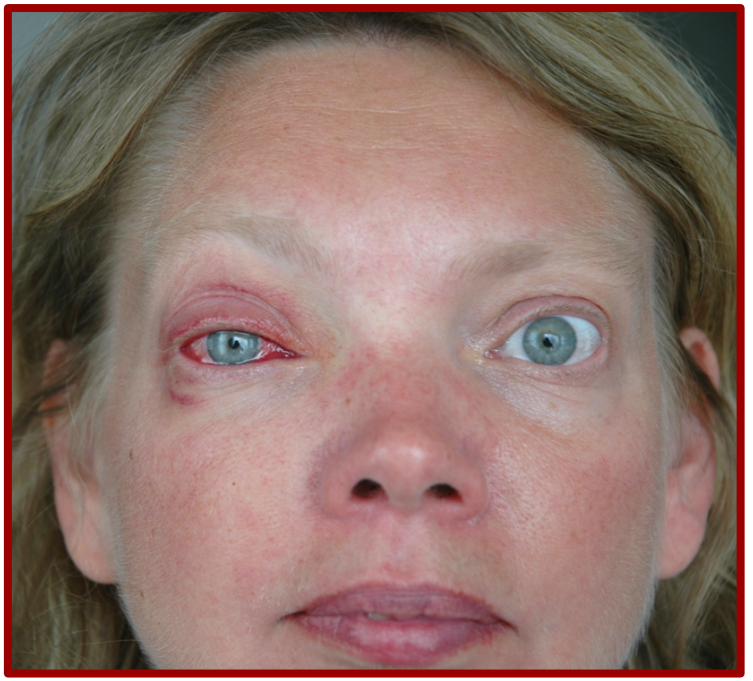
Adenoviral Conjunctivitis (Ad-Cs)

- Conjunctivitis (Cs) is a prevalent condition, comprising a significant proportion of walk-in patients to many eye-care clinicians (OD/MD)
- Adenovirus (Ad) is a particularly contagious cause of Cs
  - Can remain infectious in a dessicated state for weeks at room temp (Chaberny et al., 2003)
- Starts in one eye but moves to the second eye in a majority of cases (Cheung et al, 2003)



Ad-Cs Outbreaks

- Outbreaks can occur wherever people congregate: workplaces, school, military, health care centers, etc

- Nosocomial spread significant public health issue; 3 examples:
  - 17% of 145 cases (Mueller, Klaus 1993)
  - 44% of 192 cases (D'Angelo et al., 1981)
  - 85% of 132 cases (Colon 1991)

- %'s above were # originating at place of eye exam
  - Great practice builder!!

Economic Burden

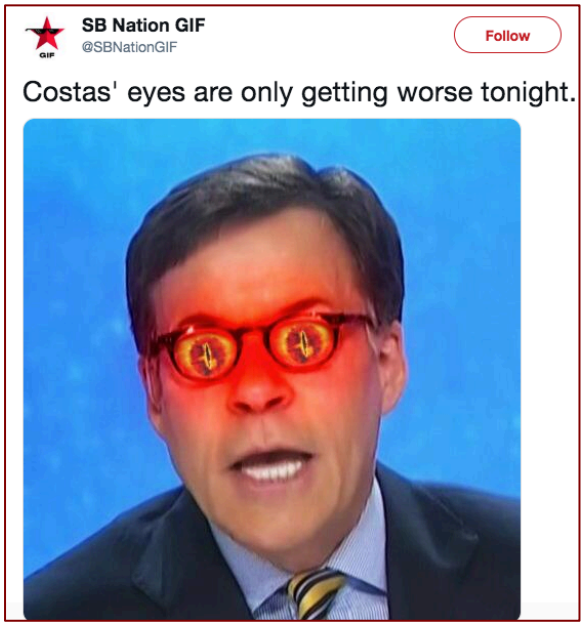
- A study in 2008 estimated that Ad-Cs costs the US economy about $670 million in its management, incorporating in work days missed (Udeh et al., 2008)
  - Reported average to be 5 days missed (range 2-10)

- Specific example (Doyle 1989):
  - Outbreak of viral conjunctivitis in microelectronics factory (145 cases among 350 workers)
  - Estimated cost close to a million dollars due to work days lost, temporary employee costs and overtime

- Can disrupt health care delivery

Ad-Cs Morbidity

- Highly symptomatic
  - Bulbar redness, pain, itching, tearing, discharge, marked lid swelling, photophobia, foreign body sensation and decreased vision during the infection



- Typically self-limiting
  - Minority develop corneal infiltrates that coalesce and cause permanent vision loss (Ford et al., 1987)

Ad-Cs Treatment

- Effective treatment approach could decrease the time-course of the condition, limit transmission to other individuals and/or also improve the symptom burden
- Currently, no FDA-approved treatments for Ad-Cs
- A treatment that reduces the contagious period by even one or two days could have significant public health and economic impact.
- Current standard of treatment is symptomatic with artificial tears, antihistamines and cold compresses

Ad-Cs Treatment

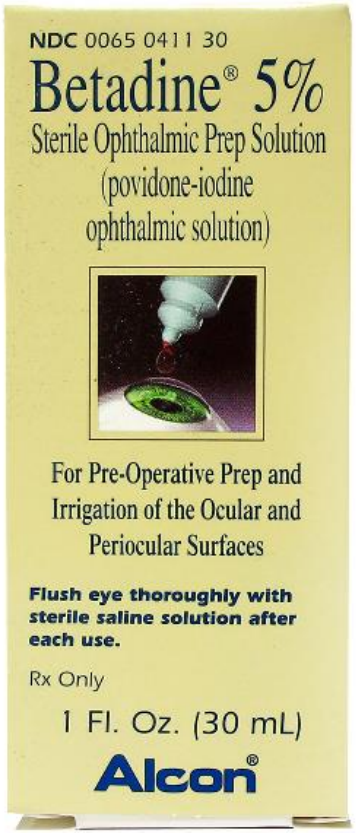
- Antibiotics – doesn't target the underlying etiology
  - Low incidence of co-infection; microbial resistance
- Steroids – can be adjunctive therapy with others
  - Indicated when infiltrates, membranes, uveitis present
  - On its own, linked to increase in viral replication
- Topical anti-virals – some promising results but no compound has established efficacy thru controlled RCT
  - Ganciclovir appears to inhibit viral replication *in vitro*
- Interferons may be prophylactic to boost immune system

Antiseptic: PVP-I

- Povidone-iodine (PVP-I) works by iodination and oxidation of cytoplasmic and membrane compounds

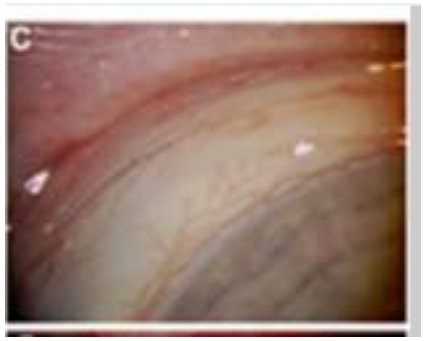
- Broad-spectrum – in addition to adenovirus, there is evidence for effectiveness against:
  - bacteria (no microbial resistance), herpes simplex, Chlamydia and enteroviruses (Reimer et al., 2002)

- Used as a surgical scrub for over 50 years, commonly used to prevent infection during ocular surgeries

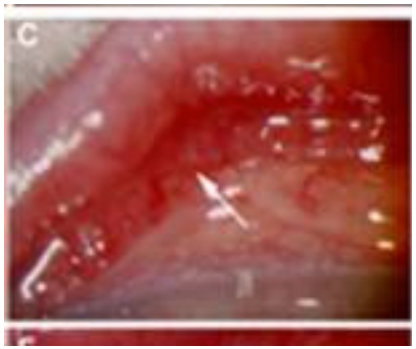


- *In vitro*, it is very effective at killing free Ad, less so against intracellular Ad (Monnerat et al., 2006)

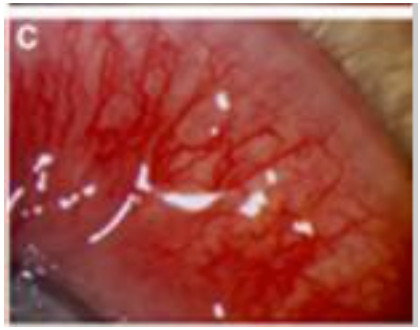
- However, in a rabbit model of Ad-Cs, PVP-I (0.4% with 0.1% dex) significantly improved the clinical sign scores and reduced extracellular viral titers (Clement et al., 2011)



Tx: PVP-I/Dex
7 days



Tx: Cydofovir
7 days



Tx: Tobra/Dex
7 days

PVP-I

- Ideally, a treatment is not only effective, but it is safe, low-cost and widely accessible

- PVP-I has been used for decades as topical antiseptic on neonates, children, adults

- Ophthalmic PVP-I formulation (Betadine® 5%, Alcon) is FDA- approved for “the prepping of the periocular region and irrigation of the ocular surface”

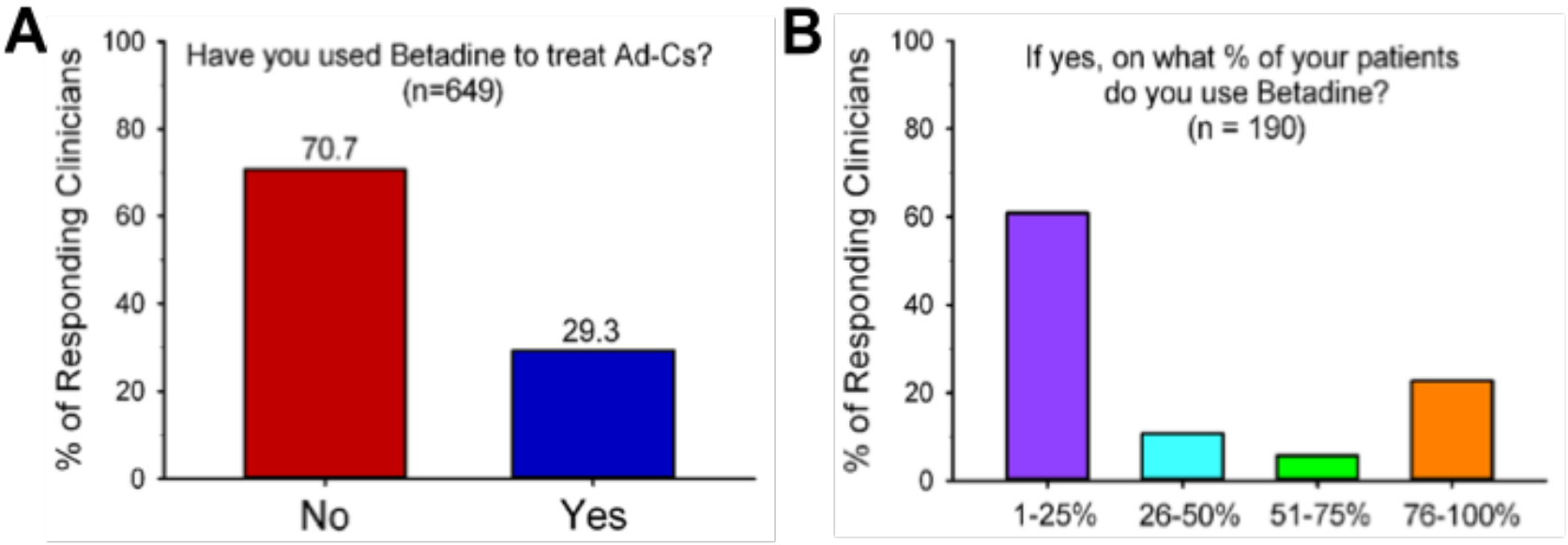
- The cost per 30 ml single-use package of 5% PVP-I ophthalmic solution is about $21, compared to $140 per 5gm tube of Zirgan™ (gancyclovir).

- PVP-I is widely available in developing countries where it can be prepared from powder or stock solutions meant for other antiseptic purposes.

- Off-label use of PVP-I for Tx of Ad-Cs has gained credence over the last decade in influential reviews and editorials…

- For example…
- In the widely disseminated, annually-published ‘Clinical Guide to Ophthalmic Drugs’, Melton and Thomas reported that “...a one-time application of povidone-iodine should be sufficient for alleviating the condition”
- A few years ago, a group of clinicians/researchers at various optometry schools/practice settings were curious about how wide-spread PVP-I’s use was
- We surveyed OD’s and MD’s at seven clinical conferences using ‘clickers’ or paper surveys

Clinical Use of PVP-I for Ad-Cs



Than T, Hartwick A, Shorter E, Lonsberry B, Gordon M, Freddo T. How to manage adenoviral conjunctivitis. 2014. *Optometry Times* Feb 18

- A significant minority of eye care providers report using PVP-I for Ad-Cs

Randomized Trial Assessing PVP-I for Ad-Cs

- Therefore, a well-designed randomized controlled trial that tests (validates or disproves) the usefulness of this growing practice was deemed likely to impact clinical practice, regardless of the results

- A positive trial-outcome of a single, in-office treatment of 5% PVP-I could revolutionize management of Ad-Cs, especially in developing countries where external eye infections are endemic

- The absence of effect would spare hundreds of thousands from ineffective treatment. Either result would provide a rational basis for health insurance plans

- The Reducing Adenoviral Patient-Infected Days (RAPID) Study

Started with 6 clinical sites, finished with 9

- Big decisions regarding study inclusion:
  - 1) How far along in the disease for cutoff point?
    - Short cut-off (i.e. 1 day after symptom onset) and recruitment is difficult
    - Long cut-off (i.e. 7 days) and disease may be near resolved; makes it difficult to find treatment effect
  - 2) We wanted only Ad-Cs patients randomized to treatment. How to determine whether the pink eye was truly Ad-Cs?

Cut-Off Point for Symptom Duration

- We used a cutoff of 4 or less days since symptom onset
- So, if subject woke up on Sunday with a pink eye, then they could enroll in the study if they came on the Thursday or earlier
- If they presented on the Friday, they were not screened for the study
- A caveat to the study design is that it relied on subject-self-report for symptom onset

Diagnosis of Ad-Cs

- Timely and accurate diagnosis of Ad-Cs at first visit crucial to the success of this clinical trial

- Only subset of acute Cs cases have adenoviral etiology
  - Proportions vary greatly (ranging from 5 to 62%) based on confirmed Ad-Cs

- Meta-analysis concluded bacterial & viral Cs cannot be reliably distinguished clinically (Reitveld et al., 2003)

Diagnosis of Ad-Cs

- In cases clinically diagnosed as Ad-Cs, concordance with molecular testing is as low as 8% (range 8 to 82%)

Harding et al. 1987 Weiss et al. 1993; Gigliott et al. 1981; Woodland et al. 1992; Fitch et al. 1989; Saitoh-Inagawa et al.1996

- Why such a range?

- As a whole, data speaks to the challenge of correctly diagnosing Ad-Cs at 1st visit

Laboratory Diagnostic Techniques

- The gold standard for confirming Ad-Cs is to test conjunctival swab samples using cell culture or polymerase chain reaction (PCR) techniques

- PCR is probably the most used definitive test currently. An advantage is that it can provide a quantification of the viral titers present in the sample (qPCR).

- Disadvantage is that the sample usually has to be sent out to a lab for the testing – takes time
  - Can’t be used to make treatment randomization decisions on the first visit

Adenoviral Immunoassay

- The AdenoPlus™ test was a point-of-care immunoassay
- Uses monoclonal antibodies raised against the hexon protein that is conserved in all known serotypes, and yields a bivariate “yes/no” result for presence of Ad
- Major advantage is result obtained within 15 minutes
- For clinical trials on Ad-Cs, the rapid diagnostic result facilitates treatment randomization at first visit

Adenoviral Immunoassay


QUIDEL
25 Tests
QuickVue
hCG Urine TEST
QUIDEL
QuickVue
hCG Urine TEST
25 Tests
ITEM
QuickVue
QUIDEL Corporation San Diego CA USA
Procedure Card
QuickVue
hCG Urine TEST
ENGLISH
QuickVue
CLIA Complexity: WAIVED
INTENDED USE
SUMMARY AND EXPLANATION
PRINCIPLE OF THE TEST
TEST PROCEDURE
INTERPRETATION OF RESULTS

Adenoviral Immunoassay

- The first publication that evaluated the performance of the AdenoPlus immunoassay reported high positive (94%) and negative (95%) predictive values for the device (Sambursky et al., 2013)

- We decided to use Adenoplus as screening tool for eligibility:
  - Positive AdenoPlus enable treatment randomization
  - Negative AdenoPlus – patient completed baseline visit (including conjunctival swab) but no Tx or F/U

- Conjunctival swabs were obtained from screened subjects and PCR was done on samples

Adenoviral Immunoassay

Eye was anesthetized with proparacaine

After 5 min, sampling fleece was dabbed temporally on the inferior palpebral conjunctiva and dragged nasally

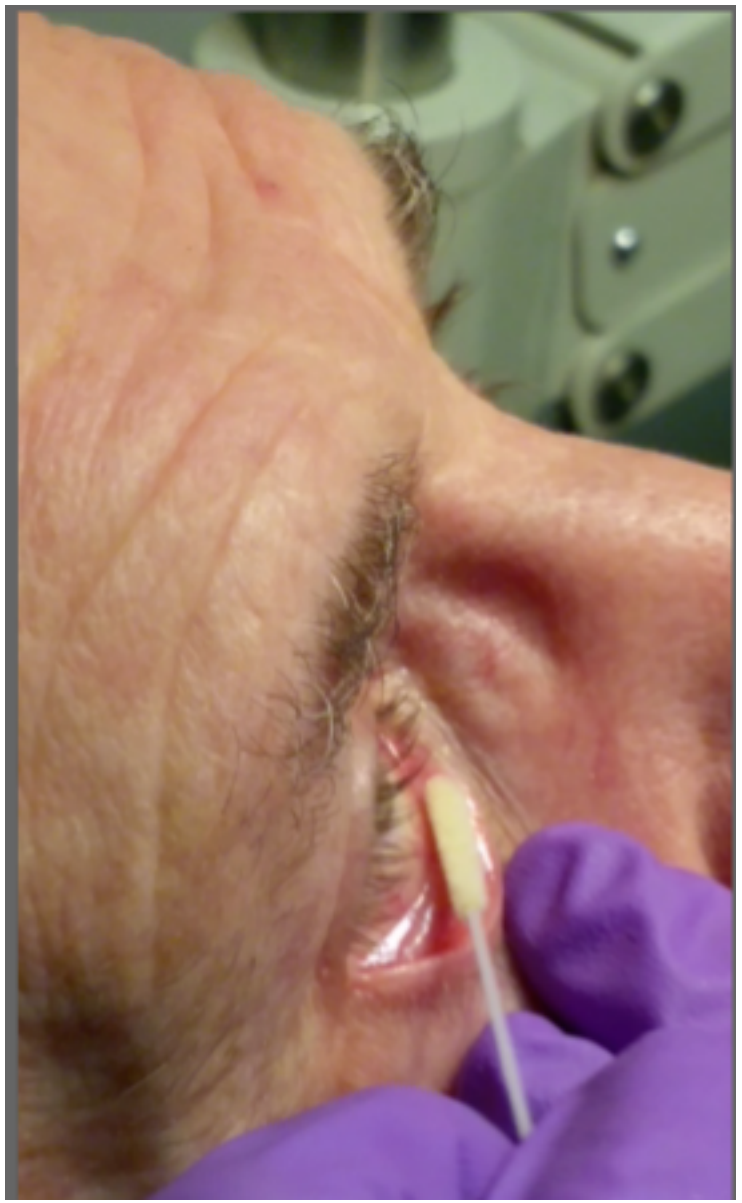
Process was repeated 8 times, with the fleece resting against the nasal palpebral conjunctiva for 5 s at the end

Fleece collector placed in the test cassette body and the absorbent tip was immersed in the supplied vial of buffer for 20 s

Test results were read after 10 min

Conjunctival Swab

- Swab of inferior palpebral conj was obtained
- Immersed in viral transport medium
- Vial placed on ice and
- Frozen at -80°C within 3 h
- Shipped to Wash U, St. Louis for PCR 1-2x/year



Double-Masked Randomization Treatment Trial

- If AdenoPlus came back positive….
- Subject received proparacaine and then either 5 drops of 5% PVP-I or artificial tears. Lid margins were rubbed with Tx-soaked swab. After 1 min, eye was saline lavaged
- Masking of subject to treatment was attempted
- Discharged with PF artificial tears qid for 7 days
- Returned for F/U visits from masked examiner at Days 1-2, 4, 7, 14, 21

PVP-I Application

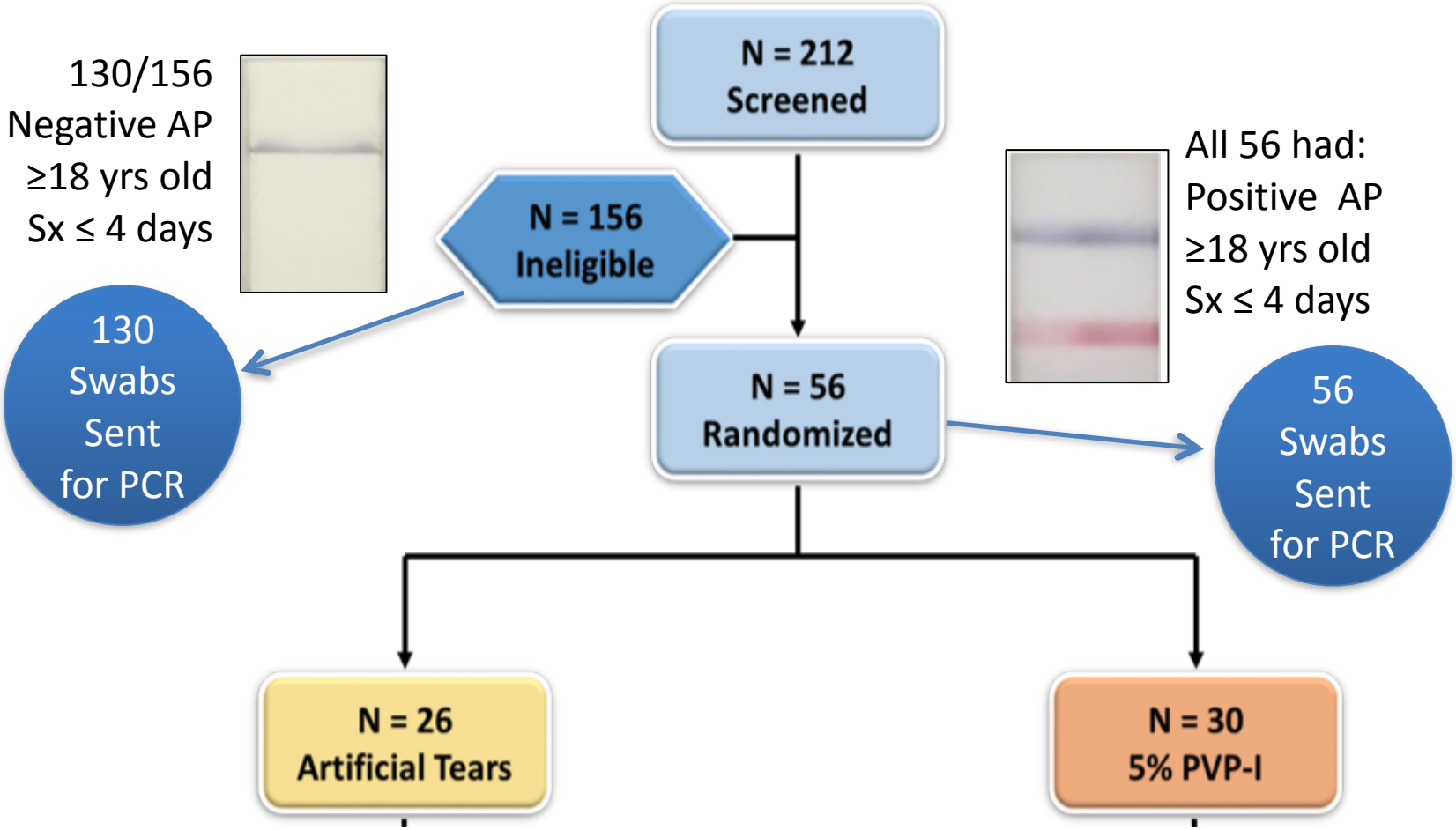
Inclusion Criteria

- Age ≥ 18 years
- Symptom onset ≤ 4 days
- Positive AdenoPlus™

Exclusion Criteria

- History of thyroid disease
- Allergy to iodine or study medications
- Ocular surgery within the past 3 months
- Skin vesicles
- Corneal dendrite, infiltrate, ulcer, abrasion or foreign body
- Conjunctival membrane or pseudomembrane
- Anterior chamber inflammation
- Pregnancy/nursing

- Subjects with red/pink eye screened in 9 clinical sites

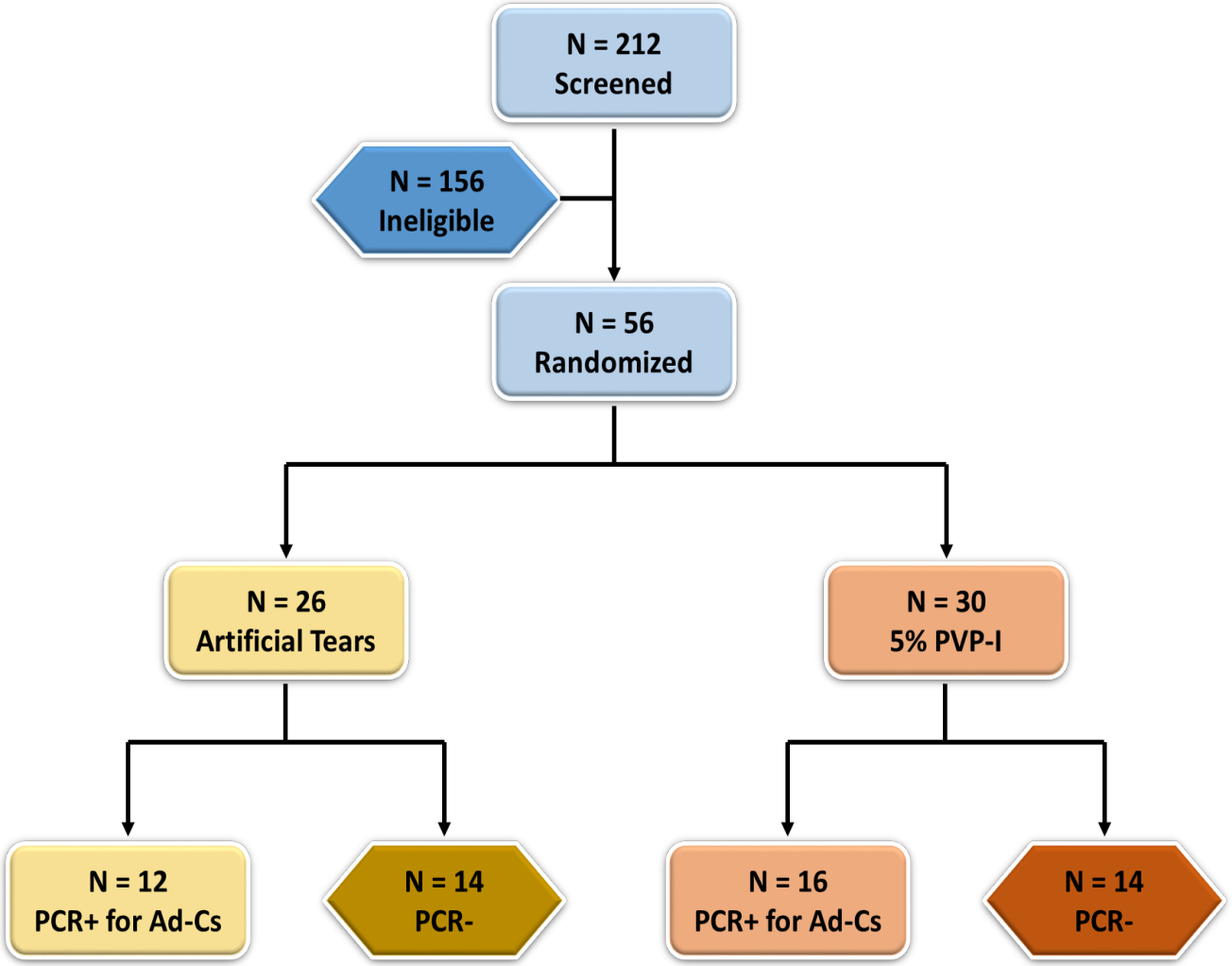


Predictive Value of the AdenoPlus

	PCR +	PCR -	**Total**
AdenoPlus +			
AdenoPlus -	2	128	130
Total			

- Negative Predictive Power = 128/130 = 98.5%
- Positive Predictive Value = 28/56 = 50%
- With PCR as comparator; Sensitivity = 93%, Specificity = 81%

Randomized Ad-Cs Subjects



End Result: 12 Ad-Cs in Tears; 16 Ad-Cs in PVP-I

Point-of-Care Immunoassay

1: Take a Sample

2: Assemble the Test

3: Run the Test

4: Read and Interpret

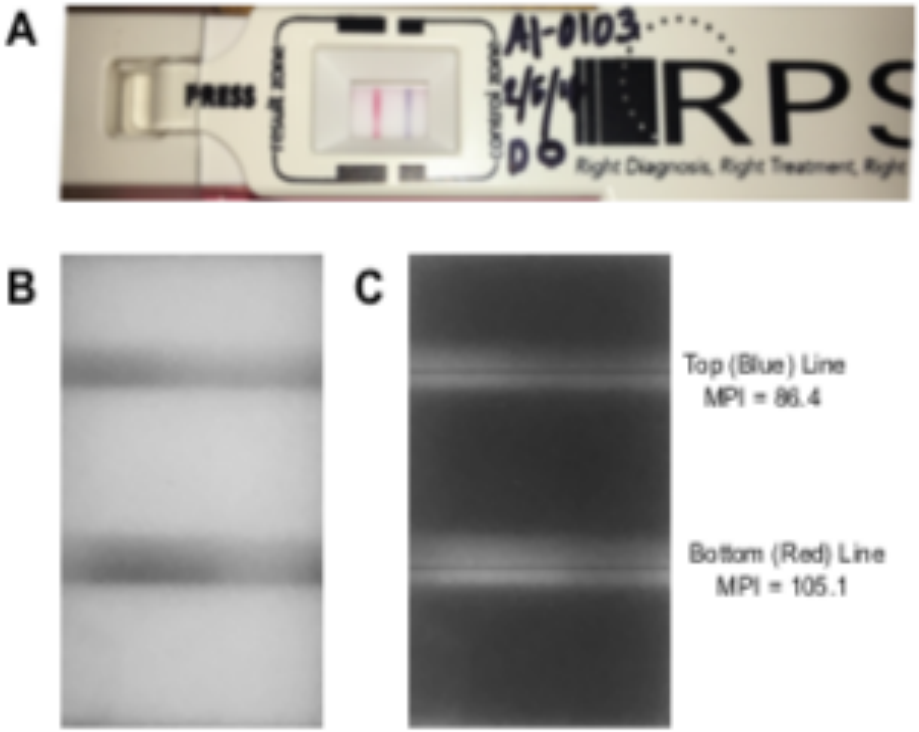
Immunoassay Densitometry

tvst

Article

Predictive Accuracy and Densitometric Analysis of Point-of-Care Immunoassay for Adenoviral Conjunctivitis

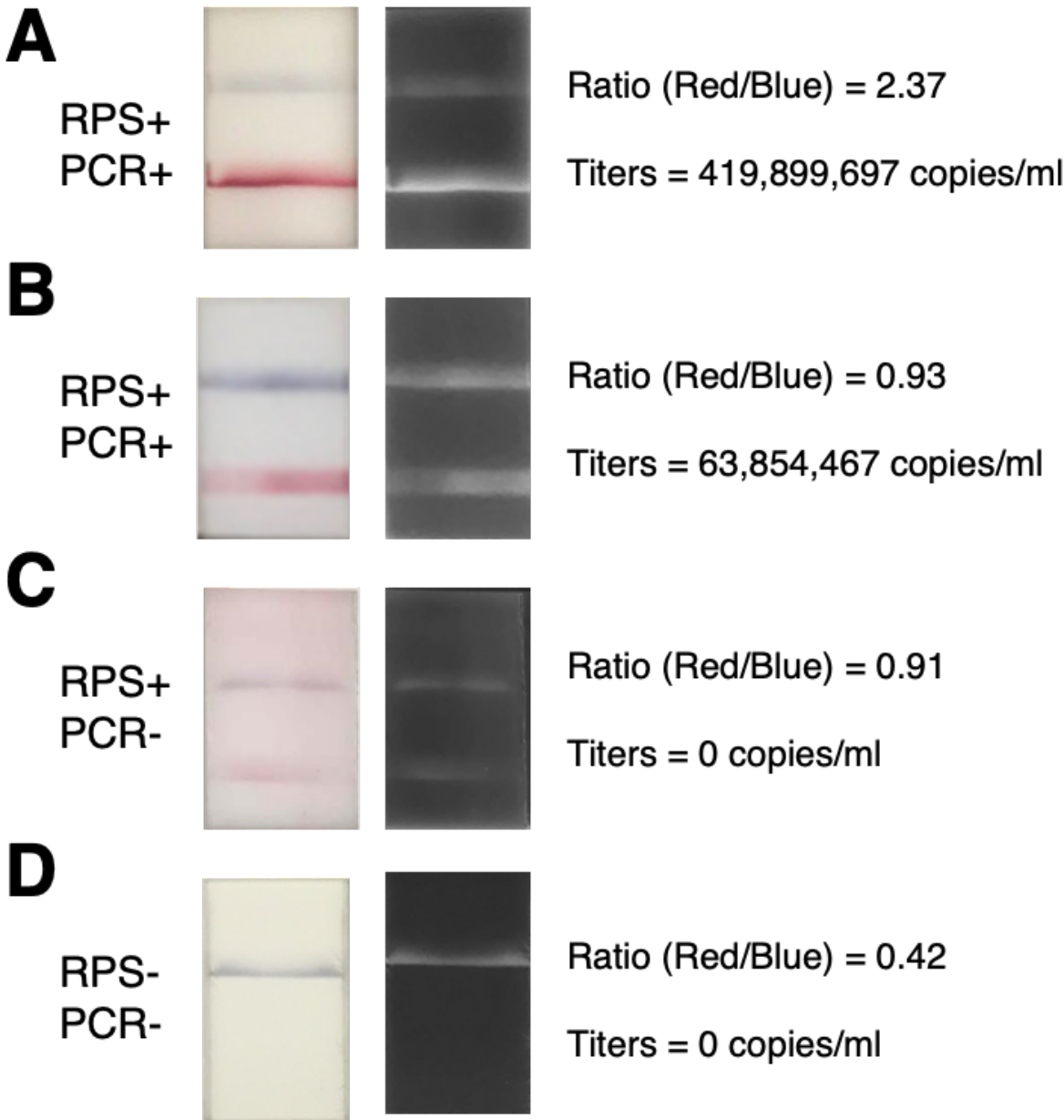
Spencer D. Johnson[1], Jennifer S. Harthan[2], Tammy Than[3], Mary K. Migneco[4], Ellen Shorter[5], Meredith M. Whiteside[6], Christina E. Morettin[2], Christian K. Olson[7], Crystal A. Rosemann[7], Mathew S. Margolis[4], Leonard W. Haertter[4], Julia B. Huecker[4], Bojana Rodic-Polic[8], Richard S. Buller[4], Gregory A. Storch[4], Mae O. Gordon[4], and Andrew T. E. Hartwick[9]



The red-to-blue band densitometry ratio here is: (105.1/86.4) = 1.22

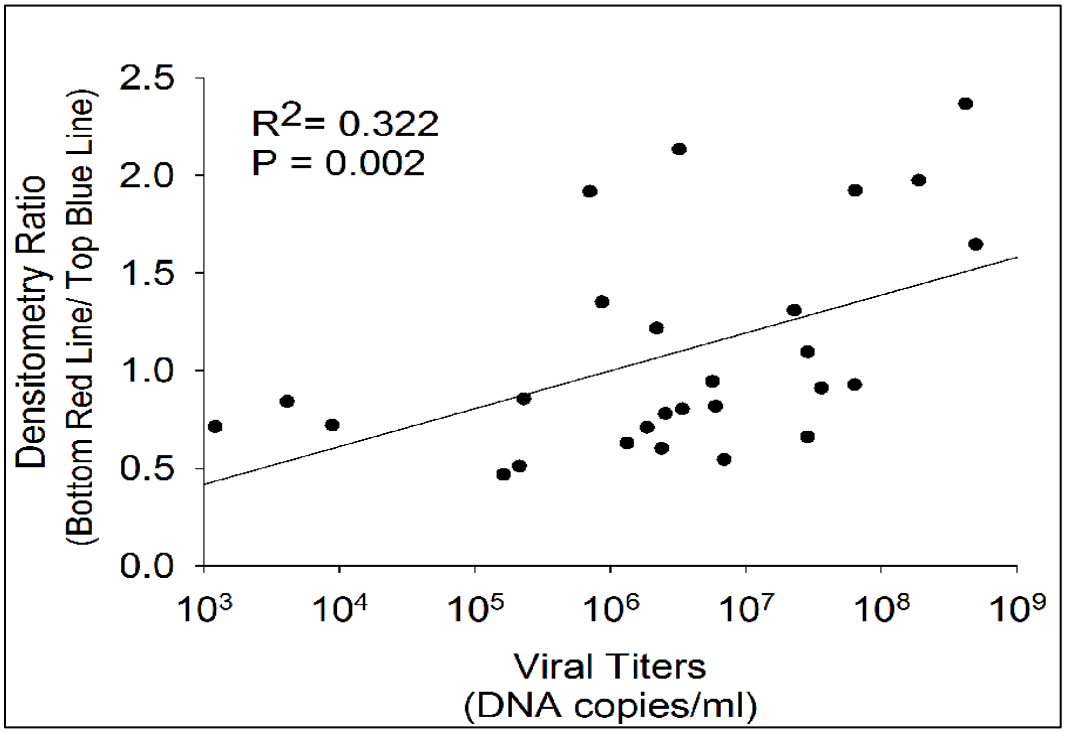
AdenoPlus Densitometry

- Most of the AdenoPlus tests were photographed (cell phone)
- Measured ratio of band intensity to compare on PCR positive versus PCR negative results



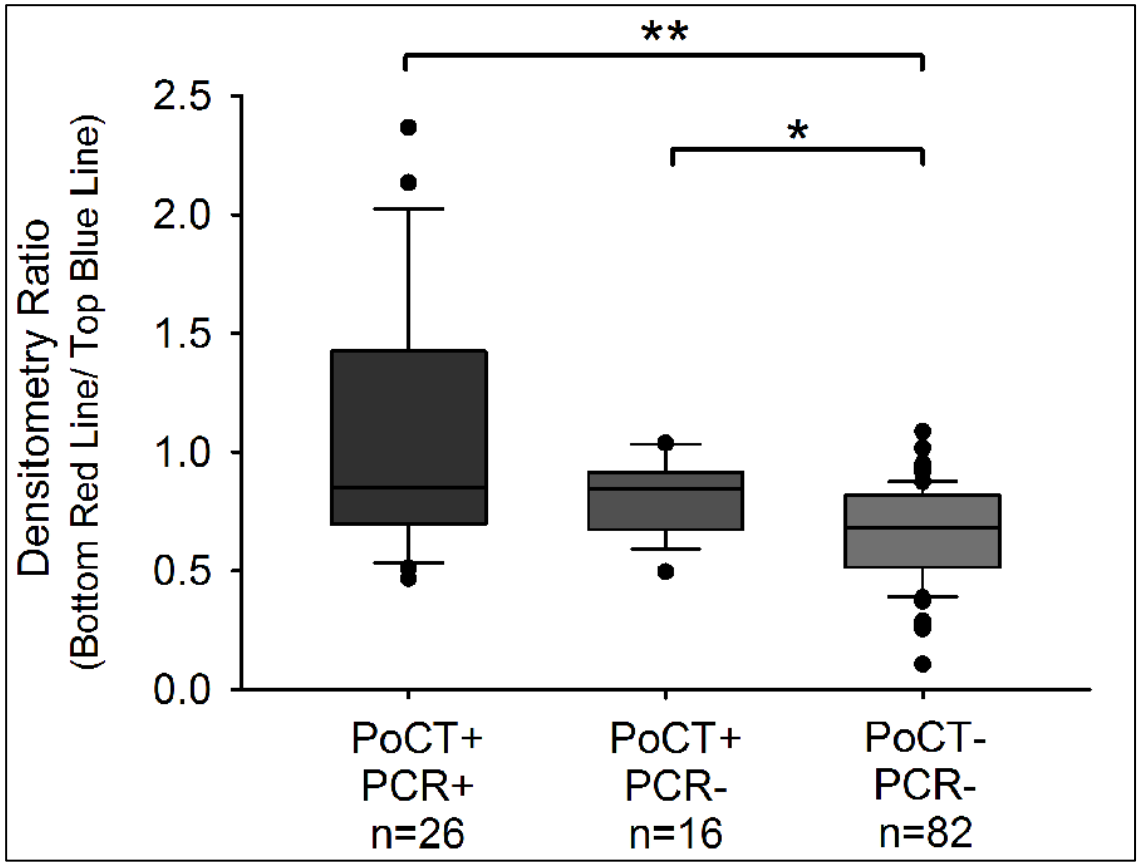
AdenoPlus Densitometry

- Correlation of viral titers to densitometry ratio in samples that were RPS+ & PCR+ (n = 26)



- Interpretation: the brighter the red band, the higher the viral titers on subsequent PCR analysis

- Box plot (median and percentiles)

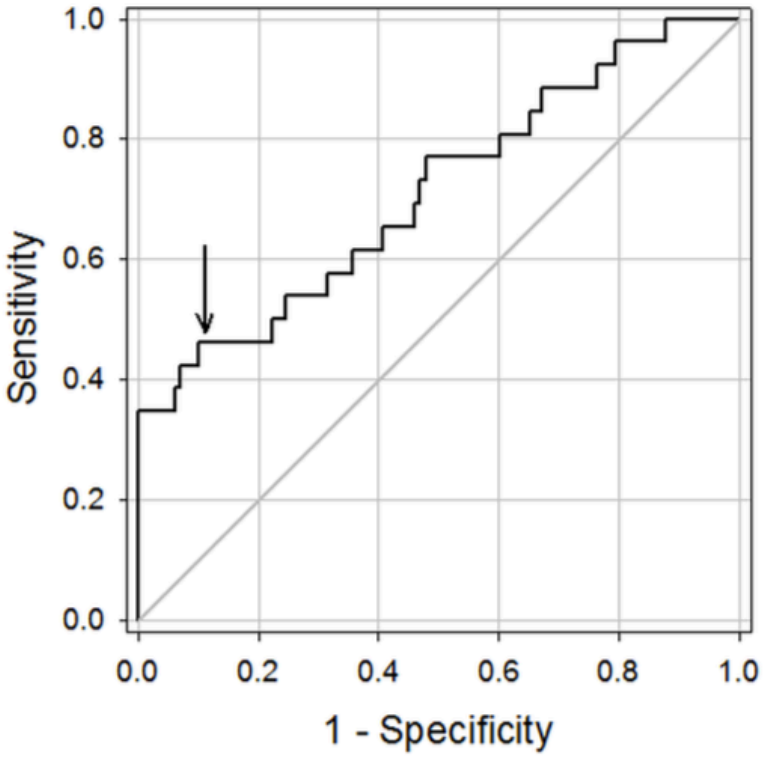


Note rarity of PCR- ratios greater than ~ 0.9

Clinical pearl:
If red line is brighter than the blue line (ratio >1), you can be more sure it is Ad-Cs

Dunn's posthoc: 1 vs 2 (p=1.0); 1 vs 3 (p<0.001); 2 vs 3 (p=0.019)

ROC Curve



Area under curve was 0.71 for the diagnosis of Ad-Cs using densitometry ratios (n=124 ratios; n=26 with qPCR-confirmed Ad-Cs)

For ratio = 0.9:
sensitivity = 0.46
specificity = 0.89
(denoted by arrow).

Clinical Decision-Making Tree

- 1) If there is no red line, the case is highly likely not adenovirus
- 2) If the red line is brighter than the blue line, it is highly likely that it is adenovirus
- 3) If the red line is fainter than the blue line, you can't ascertain with any degree of certainty if it is adenovirus

Take-Away Points

- True Ad-Cs is probably more rare than you think
  - There were 212 red/pink eye patients screened for the study – some bias in selection as obvious allergy, trauma, bacterial were not screened
  - Only 30 had PCR-confirmed Ad-Cs
- If the AdenoPlus is negative, you can be about 99% sure that it is not Ad-Cs
- If AdenoPlus positive, flip a coin, 50/50 whether subject was truly Ad-Cs (perhaps darker red line improves odds)
- Recruitment for an Ad-Cs clinical trial is very, very hard

- Perhaps we needed to be more lax on our disinfection protocol…..

Disinfection

- No reported study site nosocomial transmissions in study (other patients getting infected during eye exam)
- We had a strict disinfection regime:

Compliance with CDC Guideline for Disinfection and Sterilization in Healthcare Facilities

  - hypochlorite bleach wipes (1:10 dilution) were used to disinfect all surfaces (slit lamp, counter, door knobs) at end of exam
  - Patients were asked to wash their hands upon entering the examination room and used paper towels that were subsequently disposed of in red biohazard bags.
  - Patient signed consent forms were filed in a red folder indicating biohazard. Pens used were put in biohazard bag
  - Clinician changed gloves 3x during exam

- However, one clinical examiner contracted Ad-Cs during study!

Diagnostic Accuracy of Signs and Symptoms

Table 2. Univariate logistic regression results for candidate variables based on PCR status for adenoviral conjunctivitis at the screening visit

	PCR status				
	Negative N = 155		Positive N = 30		Univariate
	Mean	SD	Mean	SD	P-value
Self-Reported Symptoms 0 = Not at all Bothersome to 10 = Very Bothersome					
Eye tearing or watering	4.6	3.1	6.9	2.5	0.0004
Matting or discharge	4.7	3.0	6.6	2.6	0.0021
Burning or stinging	3.7	3.1	5.2	3.1	0.015
Itching	3.9	3.1	5.3	3.1	0.019
Gritty or sandy sensation	3.5	3.3	6.2	3.1	0.0002
Eyelid swelling	3.3	3.1	6.5	3.3	< 0.0001
Redness	6.9	2.6	8.9	1.4	0.0002
Blurred vision	3.3	3.1	5.0	3.5	0.011
Sensitivity to light	3.0	3.4	5.1	3.7	0.002
Overall discomfort	5.5	2.6	7.8	2.0	< 0.0001
Slit Lamp Examination 0 = Absent to 4.2 = Severe					
Lid oedema	1.1	1.0	1.8	1.1	0.0006
Eyelid matting/crusting	0.9	0.9	1.2	1.0	0.088
Serous discharge	1.6	1.0	2.4	1.0	0.0001
Mucoid discharge	0.7	0.9	0.8	0.8	0.656
Bulbar oedema	1.4	1.0	1.8	1.4	0.071
Bulbar redness	2.4	0.9	3.1	0.7	0.0006
Conjunctival follicular response	1.7	1.0	2.3	1.2	0.0019
Conjunctival papillary response	1.5	1.0	1.8	1.1	0.1498
	n	%	n	%	
Point-of-care-test positive for adenoviral conjunctivitis					
No (reference)	127	98.4	2	1.6	
Yes	28	50.0	28	50.0	< 0.0001

Diagnostic Accuracy of Signs and Symptoms

CLINICAL AND EXPERIMENTAL OPTOMETRY
https://doi.org/10.1080/08164622.2021.1984180

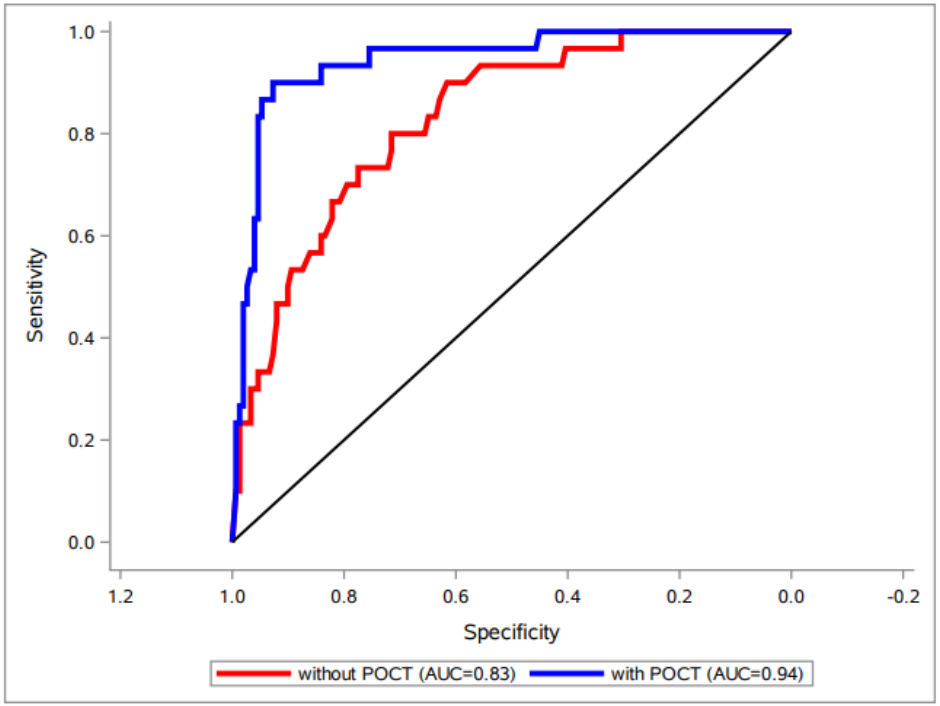
RESEARCH

Diagnostic accuracy of clinical signs, symptoms and point-of-care testing for early adenoviral conjunctivitis

Ellen S. Shorter[a], Meredith M. Whiteside[b], Jennifer S. Harthan[c], Christina E. Morettin[c], Chamila D. Perera[d], Spencer D. Johnson[e], Mary K. Migneco[d], Julia B. Huecker[d], Andrew T. E. Hartwick[f], Tammy than[g] and Mae O. Gordon[d]

AUC 0.83 best outcome (without PoCT) was to use three clinical signs:
-participant-rated eyelid swelling
-ocular discomfort
-clinician-rated bulbar conjunctival redness

AUC 0.94 with the addition of the point-of-care test



Diagnostic Accuracy

- Adenoviral Conjunctivitis correlated with more severe:
  - Ocular redness
  - Lid edema
  - Overall discomfort
- Combined with positive point-of-care test further improves diagnostic accuracy
- Improving diagnostic accuracy could prevent unnecessary work furloughs and facilitate earlier treatment decisions

Challenge: Treatment

- Big decisions regarding treatment:

- 1) Is a single, in-office treatment of PVP-I the best strategy? What about a second treatment on Day 1-2?

  2) Is the standard 5% ophthalmic PVP-I too strong? Would we get better results with a lower dose?

  3) Would adding a steroid to treatment be beneficial?

- An advantage of in-office, treatment is that patient compliance is not an issue (compared to drops of low-dose PVP-I)
- A single treatment seemed reasonable for the first study, and it was easier to keep clinicians masked
- While there is actually paradoxical evidence that lower dose PVP-I (1%) releases more free iodine than 5%, we decided to stick with the readily available version
- Similarly, we left steroids out in order to strictly test safety and efficacy of 5% PVP-I as the first study

Challenge: Follow-up Schedule

- Big question:
  - How many follow-up visits necessary? Timing?

- Follow-up schedule was hotly debated, but in the end we wanted to assess natural history of disease

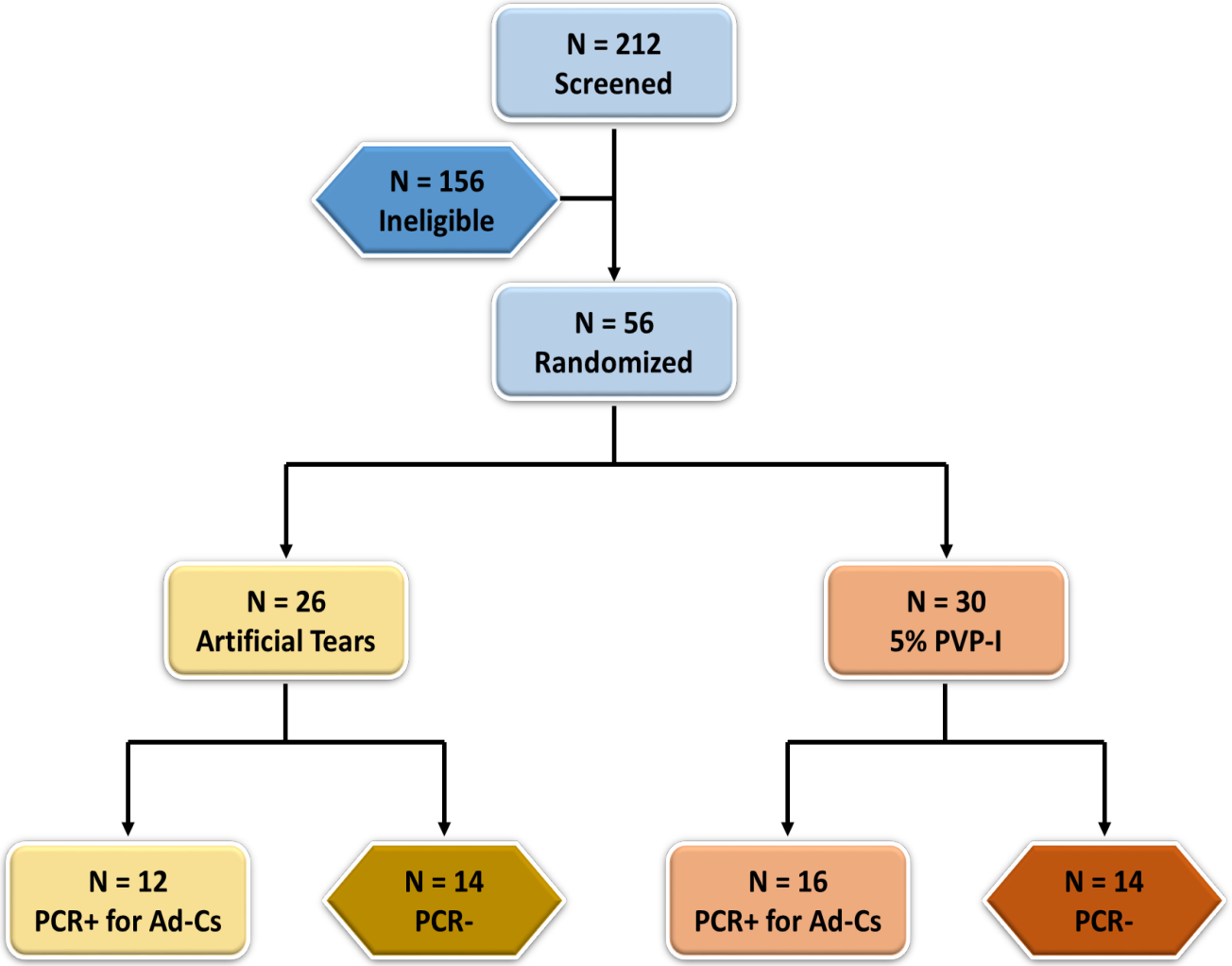
- Five follow-up visits
  - Day 1-2, Day 4, Day 7, Day 14, Day 21

qPCR-Determined Viral Titers

- Example from one subject (units are Ad DNA copies/ml)

	Raw Data	Normalized (%)
	D1-0406	D1-0406
Baseline	256341826	100.0000
1-2 days	142511131	55.5942
4-5 days	5297125	2.0664
7 days	58497	0.0228
14 day	7814	0.0030
21 day	0	0.0000

Randomized Ad-Cs Subjects



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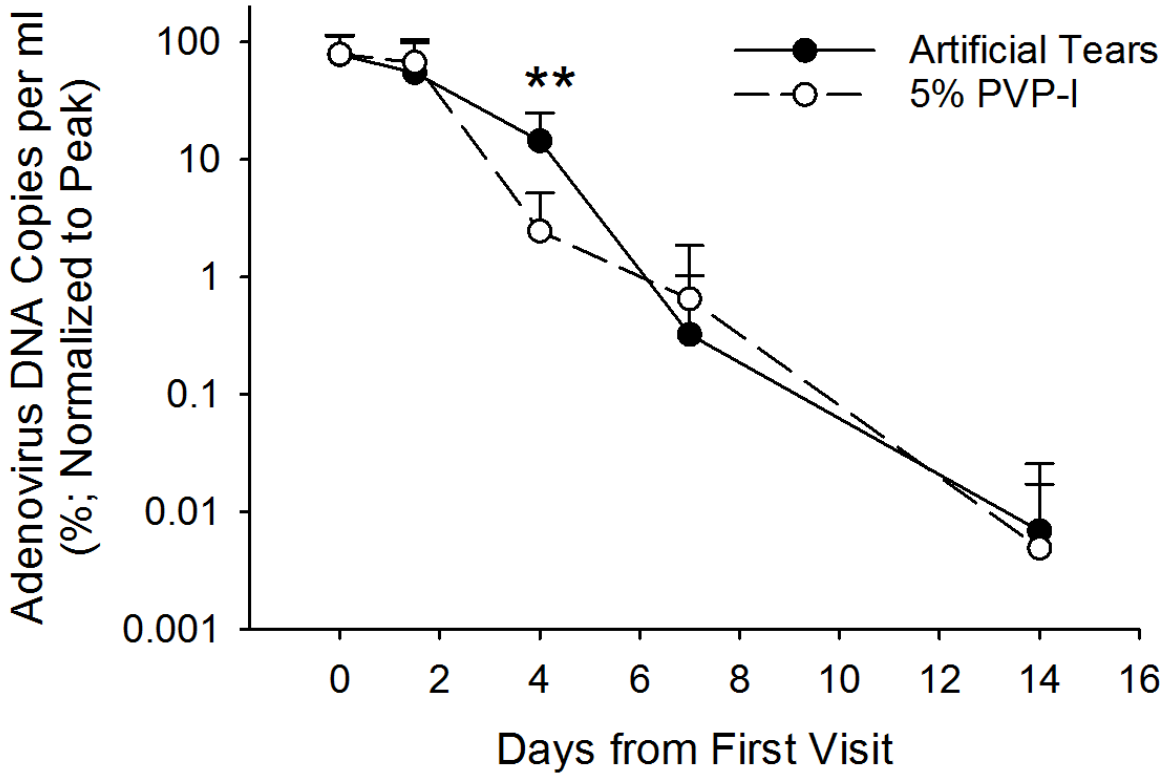
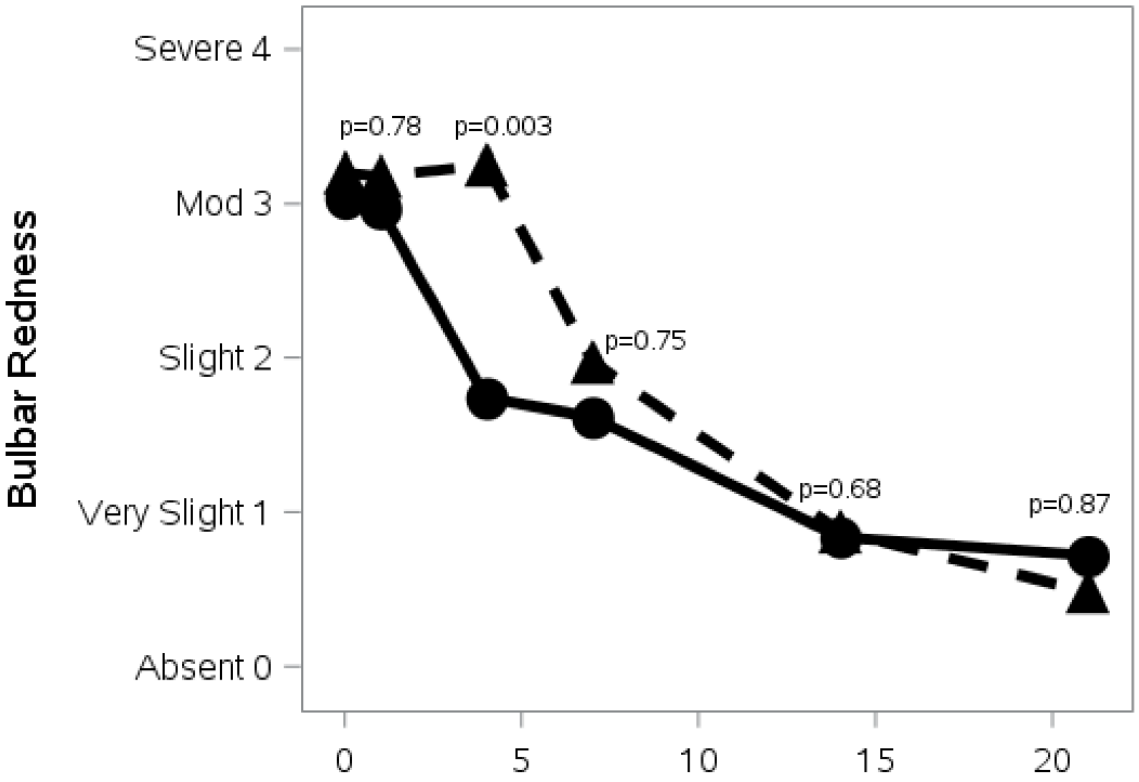


Data normalized within-individual to peak viral titers (100%) which always occurred on either Day 0 or Day1-2 visits

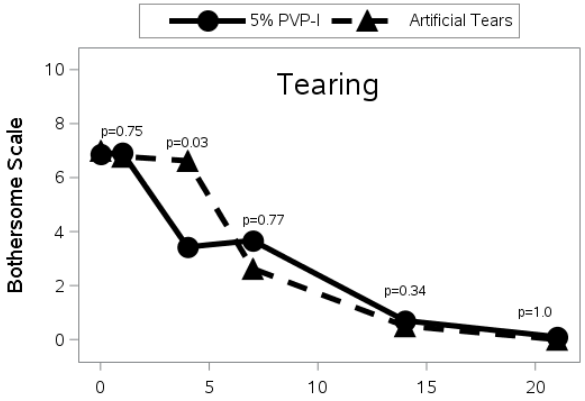
	PVP-I	Art. Tears
Day 0	78.6% (SD=36.2) n=16	77.9% (33.5) n=12
Day 1-2	67.1% (37.4) n= 13	54.8% (43.6) n=9
Day 4**	2.5% (2.7) n=8	14.4% (10.5) n=8
Day 7	0.7% (1.2) n=13	0.3% (0.7) n=9
Day 14	0.005% (0.01) n=10	0.007% (0.02) n=8
Day 21	0% (0) n=10	0% (0) n=7

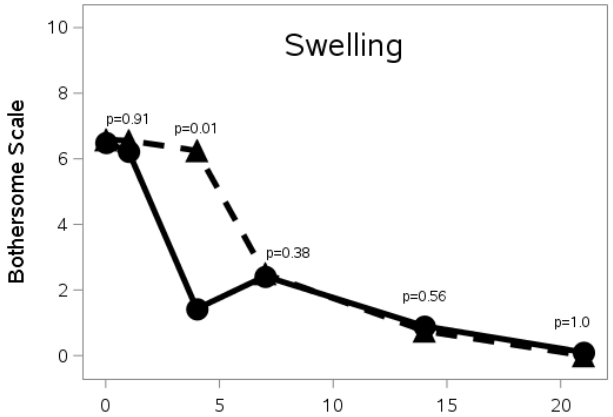
- Clinician-graded redness

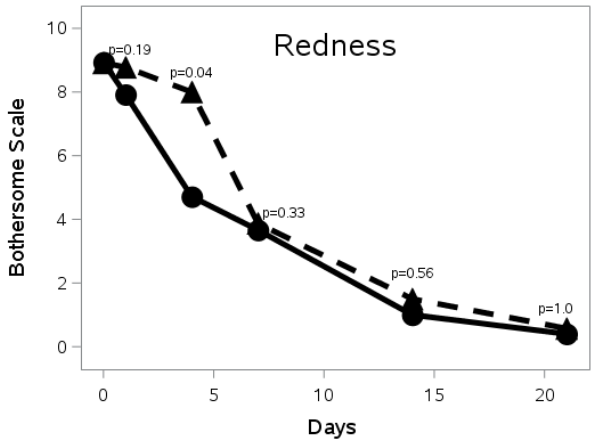


Signs/Symptoms Over Time

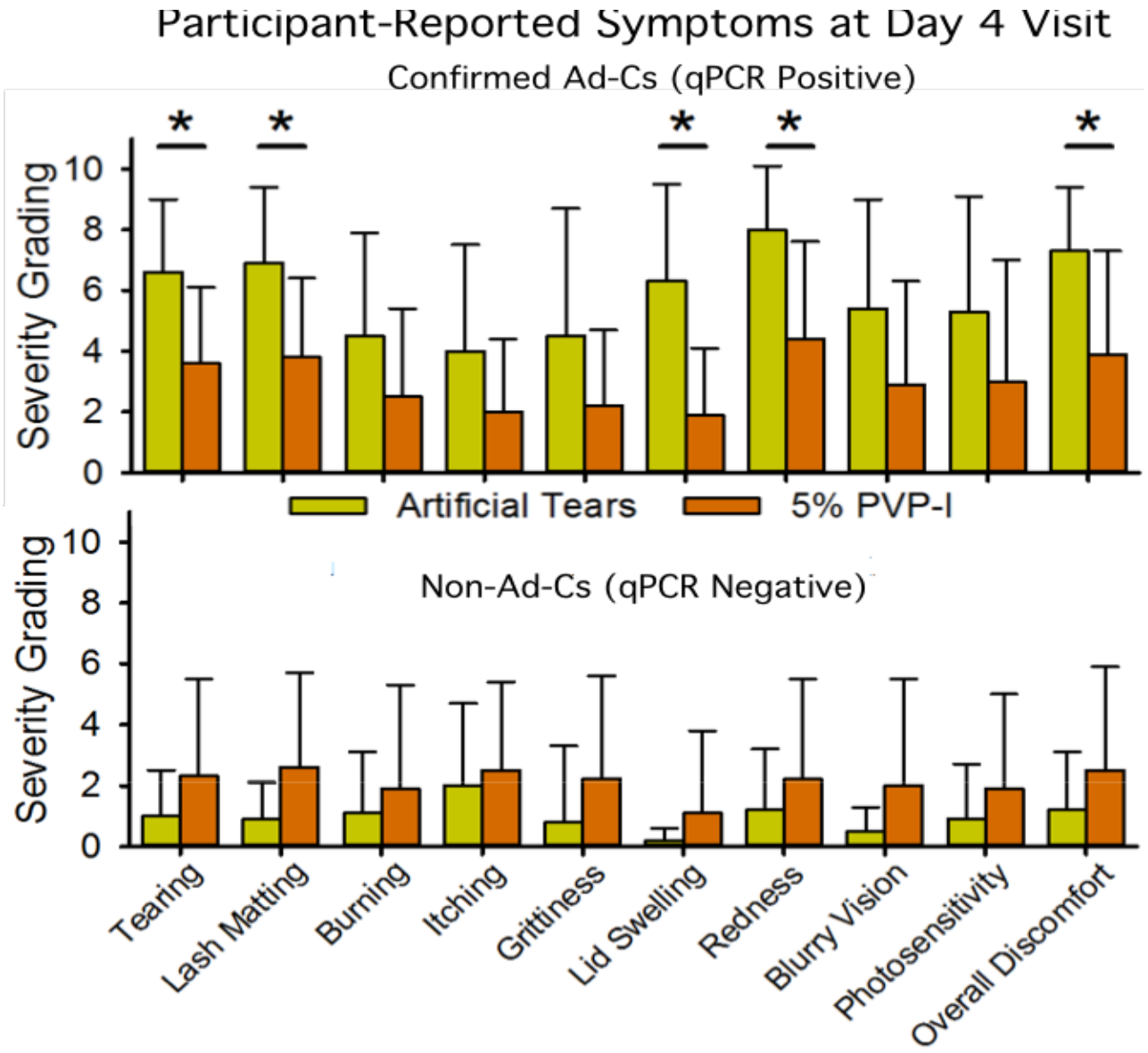
- Participant-reported symptoms



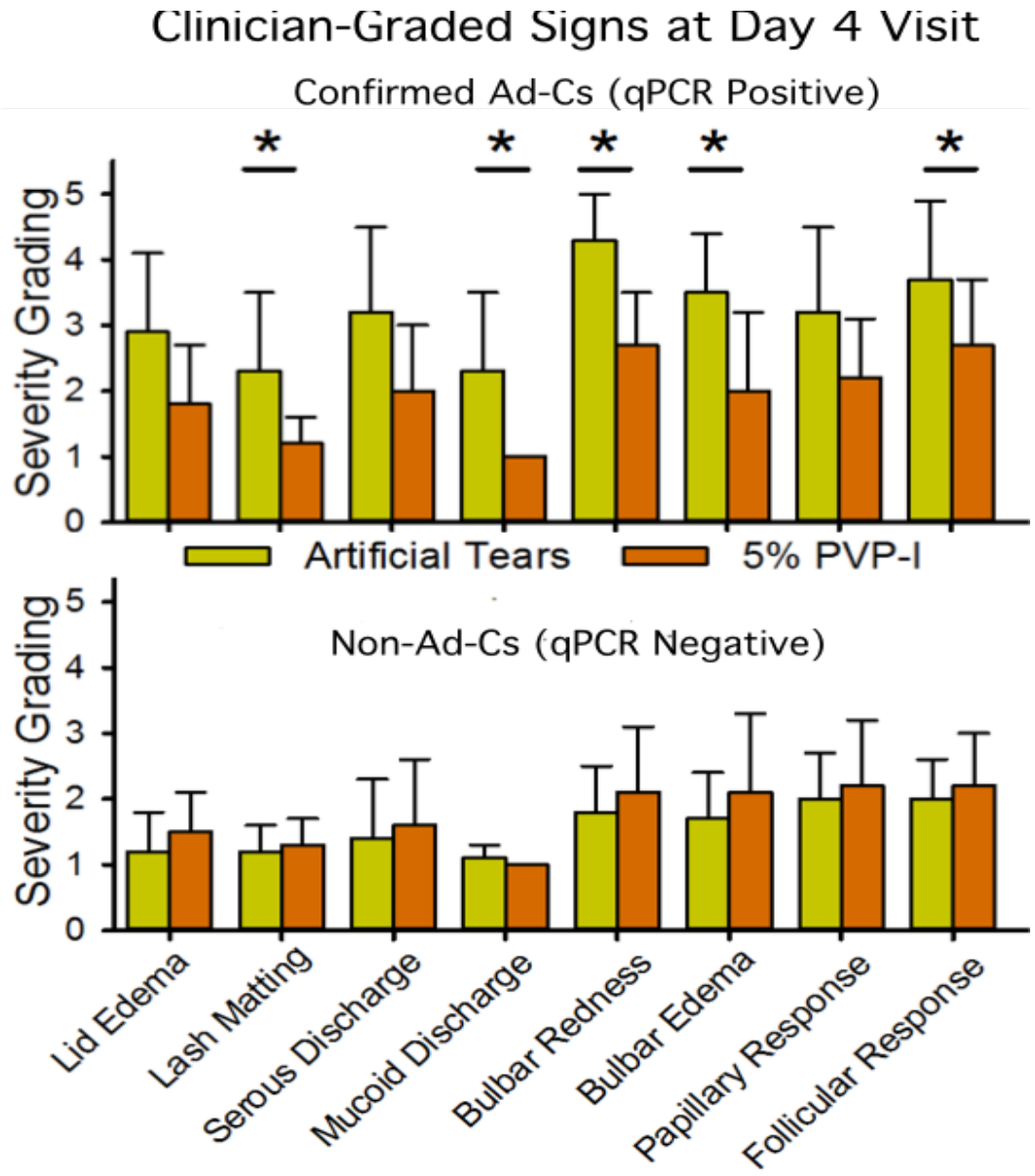




Day 4 F/U:Clinical Symptoms



Day 4 F/U:Clinical Signs



Summary of Efficacy Results

- In participants with qPCR-confirmed Ad-Cs, those receiving 5% PVP-I showed significant improvement in certain signs, symptoms and viral titers at day 4 compared to those who received AT.

- There were no significant differences between the groups in viral titers, symptoms or signs at the 1-2, 7, 14 or 21 day F/U visits in participants with confirmed Ad-Cs.

- Data provided great overview of time-course of viral titers. However, future treatment trials should focus strongly on follow-up visits between days 2 and 6

Efficacy of PVP-I for AdenoPlus+ and PCR- Cs

- In randomized participants who tested negative for Ad-Cs by qPCR, the 5% PVP-I did not significantly improve signs and symptoms at any visit with the exception of clinician-graded eyelid matting on the day 1-2 F/U visit.

- Thus, while PVP-I is supposed to be broad-spectrum, our data does indicate it is more effective against true Ad-Cs

- We are currently pursuing DNA identification approaches to try and find the etiology causing the Cs that was negative for Ad on PCR but positive on the AdenoPlus

Safety of PVP-I

- Only one reported adverse effect in PVP-I group, one subject had mild a.c reaction on the day 1 visit
  - Clinician classified it as ‘not likely related to Tx’
- At start of exam, PVP-I group rated their discomfort as 6.0±3.0 and at end it was 6.2±2.8 (P=0.78)
- At Day 1, the mean discomfort was 4.6±2.6 in PVP-I group and 5.7±2.9 in tears group, so no lingering discomfort caused by treatment the next day
- No significant diffference in corneal staining in PVP-I group versus tears

- **<u>Conclusion</u>:** These results indicate that a single, in-office application of ophthalmic 5% PVP-I is a safe treatment, and can improve clinical signs and symptoms in individuals with Ad-Cs, four days after treatment
- Whether multiple applications of PVP-I across different visits can expand the time-frame of the therapeutic effect beyond 4 days remains a question for future research.