

COMPARISON OF RAYON AND DACRON SWABS IN AMIES MEDIUM FOR *Bordetella pertussis* TRANSPORT

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ABSTRACT

Synthetic swabs (rayon and Dacron) are used to collect specimens for bacterial culture in preference to cotton swabs which may contain inhibitory or toxic substances. Regan-Lowe medium has been traditionally recommended for *Bordetella* transport. Little work has been done to determine the performance and suitability of synthetic fiber swabs used in conjunction with Amies charcoal transport medium (TM) for the maintenance of *Bordetella pertussis*. Suspensions from 20 fresh isolates of *Bordetella pertussis* were adsorbed onto rayon and Dacron tipped swabs and placed in Amies charcoal TM (Copan Italia, Brescia, Italy) and stored at room temperature. Swabs from Amies TM were plated to Regan-Lowe medium at 0, 24, 48, 72 and 96 hours. Recovery of organisms from each swab type was quantified using a plate count technique. The recovery from triplicate platings of each swab type was equivalent. Average plate counts dropped with each extra day of storage in TM. At 96 hours the counts from the rayon and Dacron swabs were 13.8% and 9.3% respectively of the 0 hours counts. We conclude that either rayon or Dacron is a suitable swab material for pertussis cultures.

THE MATERIALS

MATERIAL	DESCRIPTION	MANUFACTURER
Rayon tipped swabs	Plastic stick rayon tipped Venturi Transystem™ Amies with charcoal Sterilized by ionizing radiation Code 114C Exp. Date 30/06/00 Lot 8280	Copan Italia Brescia, Italy
Dacron tipped swabs	Plastic stick dacron tipped Venturi Transystem™ Amies with charcoal Sterilized by ionizing radiation Code 8114CQ Exp. Date 30/06/00 Lot 8171	Copan Italia Brescia, Italy
Inoculation system	BBL® Prompt™ 0.85% saline tubes Inoculation wands Code 26306 Lot 13.11F.5	Becton Dickinson Microbiology Systems Cockeysville, Maryland
<i>Bordetella</i> transport medium	Transport B. pertussis Code 2555 Exp. Date 10/01/99 Lot 2011	Quelab Laboratories Inc. Montreal, Quebec
Isolation medium	Regan-Lowe agar base Code 5072 Exp. Date 13/03/99 Lot RLAB 1201	Quelab Laboratories Inc. Montreal, Quebec
Horse blood	Horse blood defibrinated Code HD-250 Exp. Date 13/11/98 Lot 98-01019	Oxoid Inc. Nepean, Ontario

METHODS

- Using a BBL® Prompt™ inoculation wand, growth was harvested from a fresh subculture (five days incubation) of *Bordetella pertussis* grown on Regan-Lowe agar without antibiotics
- The inoculum was suspended in the BBL® Prompt™ tube of 0.85% saline
- Two further dilutions were made in 0.85% saline:
 - 1:1000 used to inoculate swabs to be tested
 - 1:50,000 used to determine the organism load applied to the swabs

SPREAD PLATE TECHNIQUE

- 0.1 mL was applied to the surface of a Regan-Lowe agar plate
- The inoculum was spread using a sterile bent wire ("hockey stick") spreader
- The surface of the plates was allowed to absorb inoculum and dry for 15 minutes
- All plates were incubated inverted in a humidified incubator @ 35°C for 7 days
- The number of colonies per plate were counted manually using a Quebec colony counter

TO DETERMINE ORGANISM LOAD

- Triplicate plate counts were performed at zero time for each culture used in the study
- 0.1 mL from the 1:50,000 dilution was applied to the Regan-Lowe agar
- After 7 days incubation, colonies were counted
- Average plate count x50 = no. of organisms delivered to swabs

SWAB COMPARISON FOR 20 FRESH CLINICAL ISOLATES OF *Bordetella pertussis*

- For each isolate 15 Copan rayon tipped swabs and 15 Copan Dacron tipped swabs were used
- 0.1 mL of the 1:1000 dilution was pipetted into a sterile 12x50 tube
- The appropriate swab was placed into the tube and allowed to adsorb the suspension
- After adsorption, the swab was placed into the Venturi Transystem™ Amies transport tube
- Triplicate platings of each swab type were performed at 0, 24, 48, 72 & 96 hours
- The swab from the transport medium was streaked evenly in three directions onto the surface of the Regan-Lowe medium to obtain a uniform inoculum
- The transport tubes were stored at room temperature pending plating
- All Regan-Lowe plates were incubated for seven days @ 35°C
- The triplicate counts from each swab type were averaged

COMPARISON WITH REGAN-LOWE TRANSPORT MEDIUM

- Two cultures were also set up in Regan-Lowe transport medium

RESULTS
AMIES TRANSPORT MEDIUM

RAYON TIPPED SWAB					
Average No. Of Colonies Per Plate @ Final Counting (N = 20)					
Average Organism Load Per Swab	0 h	24 h	48 h	72 h	96 h
1.9×10^4	525.8	203.8	138.2	90.8	72.5
Recovery (%) from inoculum	2.8	1.1	0.7	0.5	0.4
Count (%) relative to 0 hours	100	38.8	26.3	17.3	13.8

DACRON TIPPED SWAB					
Average No. Of Colonies Per Plate @ Final Counting (N = 20)					
Average Organism Load Per Swab	0 h	24 h	48 h	72 h	96 h
1.9×10^4	495.5	214.1	90.6	86	46.1
Recovery (%) from inoculum	2.6	1.1	0.5	0.5	0.2
Count (%) relative to 0 hours	100	43.2	18.3	17.4	9.3

REGAN-LOWE TRANSPORT MEDIUM

RAYON TIPPED SWAB					
Average No. Of Colonies Per Plate @ Final Counting (N = 20)					
Average Organism Load Per Swab	0 h	24 h	48 h	72 h	96 h
4.9×10^4	1380	184.5	30	6.5	3
Recovery (%) from inoculum	2.8	0.4	0.1	0.01	0.005
Count (%) relative to 0 hours	100	13.4	2.2	0.5	0.2

DACRON TIPPED SWAB					
Average No. Of Colonies Per Plate @ Final Counting (N = 20)					
Average Organism Load Per Swab	0 h	24 h	48 h	72 h	96 h
4.9×10^4	1398	197	54	8	6
Recovery (%) from inoculum	2.9	0.4	0.1	0.02	0.01
Count (%) relative to 0 hours	100	14.1	3.9	0.6	0.5

DISCUSSION AND CONCLUSIONS

- Less than 3% of the initial inoculum was recoverable from swabs in TM. The remainder of the inoculum was adsorbed into swab material or into TM
- Rayon tipped and Dacron tipped swabs showed similar results. Either could be used successfully in the transport of *B. pertussis* cultures
- Rayon tipped swabs were noted to be comparatively “harder” and did not absorb the bacterial suspensions as quickly
- On a pernasal swab, Dacron tips may be more “comfortable” for the patient and may absorb swabbed material faster
- Rayon tips may retain the integrity of the tip better
- Preliminary evidence suggests that Venturi Transystem™ Amies TM may be more effective for the transport of *B. pertussis* cultures than Regan-Lowe TM when delays in transit occur. Venturi Transystem™ Amies TM may also provide a better holding medium for *B. pertussis*
- One of the problems faced by many hospital laboratories is the infrequency of requests for pertussis culture while feeling compelled to maintain an inventory of expensive, short shelf-life pertussis TM. Amies TM is available commercially with pernasal swabs and may provide a convenient alternative.
- Earlier evidence suggested a difference between Dacron and rayon tipped swabs. We were not able to demonstrate a comparable difference with these two Copan fibers. Both fibers seem to support transport of fresh pertussis isolates.

WHERE TO GO FROM HERE

- Test Amies TM without charcoal for both culture and amplified nucleic acid detection of *B. pertussis*
- Compare Amies TM and Regan-Lowe TM with isolates from several geographic regions
- Compare Amies TM and Regan-Lowe TM with specimen containing NP flora and respiratory secretions

REFERENCES

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