

COMPARATIVE STUDY OF THE VIABILITY OF CLINICALLY IMPORTANT BACTERIA IN TWO NEW AMIES GEL TRANSPORT SYSTEMS (BD CULTURESWAB MAX V(+) AND REMEL BACTISWAB) USING NCCLS M40-A QUANTITATIVE SWAB ELUTION METHOD.

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ABSTRACT:

Careful evaluation of swab transport devices for their ability to maintain bacteria during transit to the laboratory is important to ensure delivery of quality specimens. With transit times in our facility varying from 2 - 48hrs, at room temperature, we wish to standardize on a single swab capable of maintaining both aerobic and anaerobic bacteria under these conditions. We evaluated two new Amies Gel transports; CultureSwab Max V(+), Becton Dickinson, Baltimore, MD (BD) and BactiSwab, Remel, Lenexa, KS (RL) using the Swab Elution Method described in NCCLS M40-A standard. Swabs were challenged with a combination of eight organisms both ATCC and wild strains. Swabs were inoculated with approximately 10⁶ CFU of each of the following organisms *Neisseria gonorrhoeae* (NG) ATCC 43069, *Haemophilus influenzae* (HI) ATCC10211, *Bacteroides fragilis* (BF) ATCC 25285, *Peptostreptococcus anaerobius* (PA) ATCC 27337 and clinical isolates of *Neisseria gonorrhoeae* (NGW), *Haemophilus influenzae* (HIW), *Streptococcus agalactiae* (SA), *Enterococcus faecalis* (EF). Each organism/device combination was tested in triplicate at room temperature for incubation times of 6, 24 & 48hrs. Zero time baseline counts and viability at each time point was quantified by making vortex suspensions and ten fold dilutions from each swab then culturing aliquots to perform replicate plate counts. At 6hrs holding time all swabs were able to maintain viability of all organisms tested but percent recovery differed widely from 1.2 - 96% depending on the organism and device. At 24hrs only BD was able to maintain all eight organisms with percent recoveries for NG of 4.3% with BD, 0% RL; HI 92% with BD, 0.1% RL; BF 32% with BD, 46% RL; PA 1% with BD, 0% RL; NGW 0.7% with BD, 0.35% RL; HIW 24% with BD, 7%, RL; SA >100% with BD, 29% RL and EF >100% with BD, 74% RL. At 48hrs viability was only recorded with HI held in BD >100%; BF 19% with BD, 33% RL; HIW 2% with BD, <0.1% RL; SA >100% with BD, 6.75% RL and EF >100% with both BD and RL. In our study no swab device was able to maintain all bacteria viable for 48hrs. Evaluation of transport swabs is important to understand product limitations which can be used to improve specimen management.

INTRODUCTION:

Specimen collection and transport are processes often overlooked and undervalued but are critical parts of a Quality System Model for Health Care (Fig. 1). As microbiologists we assume that commercial transport systems are engineered to meet the diverse challenges of our specimen

management logistics. Swab specimen transport and organism viability is one area of particular interest to us. Our laboratory serves The Network of The Chester County Hospital; the core of which comprises a 238 bed hospital with satellite locations in Downingtown, Exton, Kennett Square, and Lionville, Pennsylvania. At our main campus samples are transported to the lab at room temperature throughout the day for same day processing until to 10 pm at night. After 10 pm samples are held on the floors or departments at room temperature until processing the following morning. At outlying offices and satellite lab locations samples are collected from ambient temperature metal specimen drop boxes. Each office and satellite lab site has one pick-up during the day.

With our current specimen transport logistics swab samples are exposed to ambient room temperatures for as much as 2 – 48 hours. Under these transportation conditions we would like to provide our clinicians with the optimum culture swab system for maintenance of fastidious aerobes and anaerobes. Previously published studies have reported a disparity in the performance of swab transport systems when tested at room temperature. We decided to evaluate two new Amies Agar without charcoal transport swab systems; BD CultureSwab Max V(+) and Remel BactiSwab for their ability to maintain a range of aerobic and anaerobic bacteria at 20 – 25 °C. We used a quantitative Swab Elution Method for this analysis based upon the NCCLS M40-A standard.

MATERIALS:

Commercial Amies agar gel without charcoal transport swabs:

BD - CultureSwab Max V(+), Becton Dickinson, Baltimore, MD

RL - BactiSwab, Remel, Lenexa, KS

Test organisms:

Species	Abbreviation	Strain
<i>Neisseria gonorrhoeae</i>	NG	ATCC® 43069
<i>Neisseria gonorrhoeae</i>	NGW	clinical strain
<i>Haemophilus influenzae</i>	HI	ATCC® 10211
<i>Haemophilus influenzae</i>	HIW	clinical strain
<i>Streptococcus agalactiae</i>	SA	clinical strain
<i>Enterococcus faecalis</i>	EF	clinical strain
<i>Peptostreptococcus anaerobius</i>	PA	ATCC® 27337
<i>Bacteroides fragilis</i>	BF	ATCC® 25285

Figure 1.

Preanalytic				Analytic		Postanalytic		
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpretation	Results Report	Post-test Specimen Management
			X GP16	GP16	GP16 M7	GP16 M7	GP16 M7	

Adapted from NCCLS document HS1 – A Quality System Model for Health Care

METHOD: (QUANTITATIVE SWAB ELUTION METHOD¹)

Preparation of inoculum

1. Inocula of test organisms was prepared in 0.85% physiological saline (pH 6.8-7.2) to a concentration of approximately 1.5×10^8 CFU/mL (equivalent to 0.5 McFarland standard) from an 18 to 24 hour plate culture of each organism. Anaerobic bacteria required up to 48 hours of incubation to produce adequate plate growth to harvest.
2. Each inoculum was then diluted to 1:10 (1ml to 9ml) in 0.85% physiological saline (pH 6.8-7.2) solution to provide a concentration of approximately 1.5×10^7 CFU/mL.
3. The inoculum of each ATCC test organisms was prepared just prior to transferring the organism suspension to microtiter plates where the swab absorption was performed. The whole procedure did not exceed 20 minutes in order to reduce loss of organism viability in inoculum prior to incubation of inoculated swabs at the holding temperature.

Inoculation procedure

4. Each microorganism/device combination required 12 swab inoculations
 - 3 swabs for zero-time inoculum determination
 - 3 swabs for 6 hrs time point viability test
 - 3 swabs for 24 hrs time point viability test
 - 3 swabs for 48 hrs time point viability test
5. 100µl of inocula was pipetted into each of 12 wells of a microtiter plate for each microorganism to be tested.
6. Each of the 12 swabs for a particular microorganism/device combination was placed into a microtiter well until it touched the bottom and was allowed to absorb the organism inoculum for a minimum of ten seconds.
7. Sampling swabs were then placed directly into their transport device tube or container.
8. All devices were processed in the same manner. Approximately 20 minutes was taken from the preparation of organism suspension to placement of sampling devices in the appropriate transport system and placement at Room Temperature.

9. Swab transport devices were held at Room Temperature for 0, 6, 24 and 48 hrs and then processed.

Procedure for colony counting and determining organism viability

1. At the appropriate time point swabs were removed from their transport tube or container and placed it into a tube containing 1 mL of 0.85% physiological saline (pH 6.8-7.2). This is the “*primary tube vortex suspension*”.
2. Each swab was mix vigorously using a vortex mixer for a minimum of 15 seconds then as much liquid as possible is expressed from each swab by rotating it on the inside of the tube. When this process is completed for each swab then remove the swab from the tube and discard.
3. Perform three 10-fold serial dilutions (10^{-1} , 10^{-2} and 10^{-3}) in 0.85% physiological saline (pH 6.8-7.2) on each *primary tube vortex suspension*, resulting in inocula concentrations of 10^5 , 10^4 and 10^3 .
4. The *primary tube vortex suspension* was then thoroughly mixed and 100 µl of this suspension was pipetted onto appropriate plated media. A repeat pipetting step was performed to provide a second plate for duplicate determination.
5. This procedure was repeated for each of the other dilutions 10^{-1} , 10^{-2} thru 10^{-3} .
6. All spread plates for colony counts were incubated at 35-37 °C in appropriate atmosphere of incubation as described in Tables 1 and 2.



BD CultureSwab Max V (+)



Remel BactiSwab

Table 1.

Species	Inoculum (CFU)	Plate Media	Incubation T °C	Incubation atmosphere	Testing Time (hours)
<i>Streptococcus agalactiae</i>	1×10^6	5% sheep blood agar	35-37	5% CO ₂	0, 6, 24, 48
<i>Enterococcus faecalis</i>	1×10^6	5% sheep blood agar	35-37	5% CO ₂	0, 6, 24, 48
<i>Haemophilus influenzae</i>	1×10^6	Chocolate blood agar	35-37	5% CO ₂	0, 6, 24, 48
<i>Neisseria gonorrhoeae</i>	1×10^6	Chocolate agar	35-37	5% CO ₂	0, 6, 24, 48

Table 2.

Species	Inoculum (CFU)	Plate Media	Incubation T °C	Incubation atmosphere	Testing Time (hours)
<i>Peptostreptococcus anaerobius</i>	1×10^6	Brain heart Infusion agar	35-37	Anaerobic	0, 6, 24, 48
<i>Bacteroides fragilis</i>	1×10^6	Brain heart infusion agar	35-37	Anaerobic	0, 6, 24, 48

SUMMARY:

Organism A - tested against two swab devices

Inoculate each swab ► Incubate ► Vortex in saline ► Make three 10-fold dilutions

Take 100µl of primary tube vortex suspension and 100µl of each 10-fold dilution and inoculate in duplicate onto culture plates

Two swab devices at four time points and tested in triplicate = total of 24 swabs per organism test run.

RESULTS:

The protocol used in this study was based upon the quantitative Swab Elution method described in the NCCLS M40-A standard. This calls for only 0 hour and 48 hour endpoint analysis for aerobes and anaerobes and 0 hour and 24 hour endpoint analysis for fastidious bacteria like *Neisseria gonorrhoeae*. However, in our preliminary studies we failed to recover growth with certain organism/device combinations using only 24 and 48 hour endpoints so we expanded the time point analysis to include viability counts at 6 hour time point.

The ability of each swab device to maintain the viability of a particular organism was determined by comparing the average of the zero time colony counts with the growth at each time point and expressing any decrease in viability as a percentage of the zero time count. In addition, the reduction in growth was compared to zero time counts to evaluate the log₁₀ decline.

Each swab device/organism combination was tested in triplicate for each time point and colony counts of primary tube vortex suspensions and ten-fold dilutions of vortex suspensions were performed in duplicate. Minor test variation was minimized by averaging colony counts from these multiple data points. Colony counts and percent recovery is summarized in the following graphs 1 – 8.

All organisms were recovered from both transport systems at the 6 hour holding time point. After 24 hours only BD CultureSwab Max V(+) was able to maintain the viability of all eight test organisms. *Neisseria gonorrhoeae* ATCC 43069 and *Peptostreptococcus anaerobius* were not recoverable from the Remel BactiSwab after 24 hours. After 48 hours neither the BD nor the Remel transport systems were able to support the viability of *Peptostreptococcus anaerobius* or *Neisseria gonorrhoeae* strains however, the NCCLS M40-A standard does not specify maintenance of *Neisseria gonorrhoeae* beyond 24 hours. Remel BactiSwab failed to maintain the viability of both clinical strain and ATCC 10211 strain of *Haemophilus influenzae* at 48 hours.

We noted that less fastidious bacteria namely *Enterococcus faecalis* and *Streptococcus agalactiae* increased in numbers during holding times in the BD product and similarly but to a much lesser extent with *Enterococcus faecalis* in the Remel product.

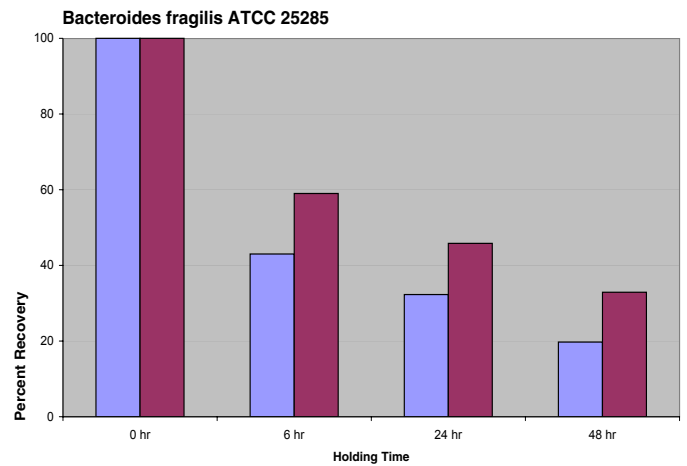
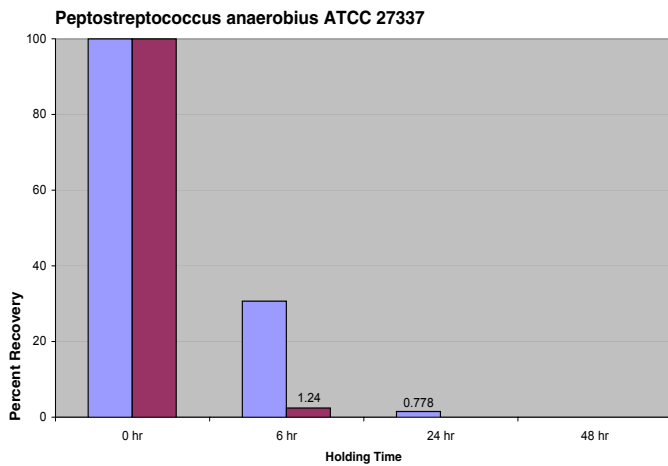
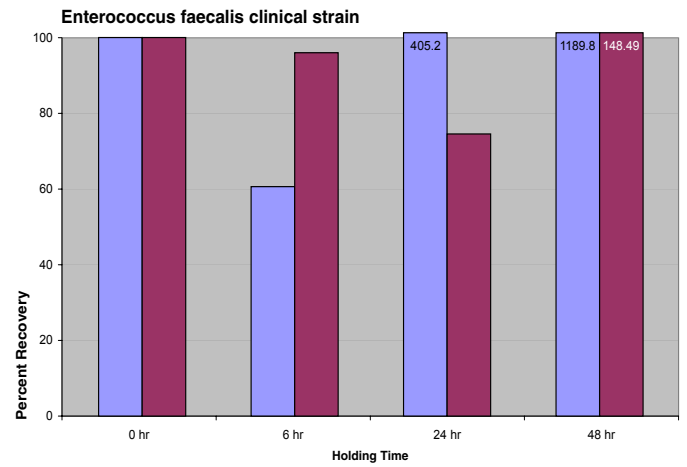
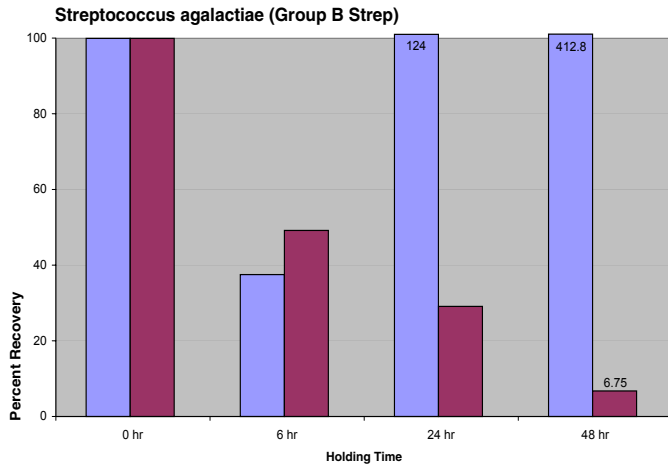
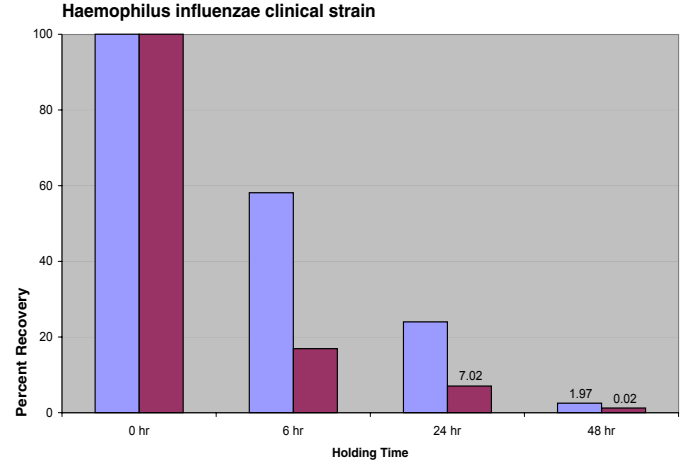
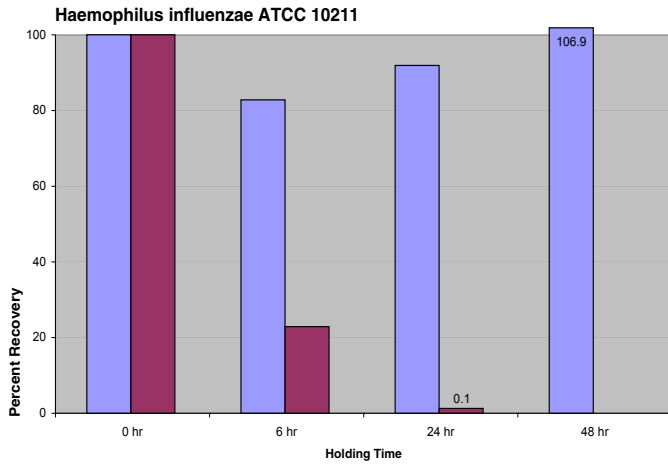
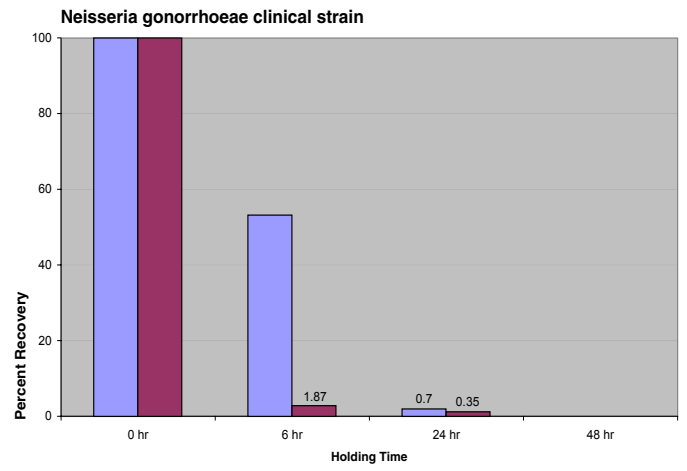
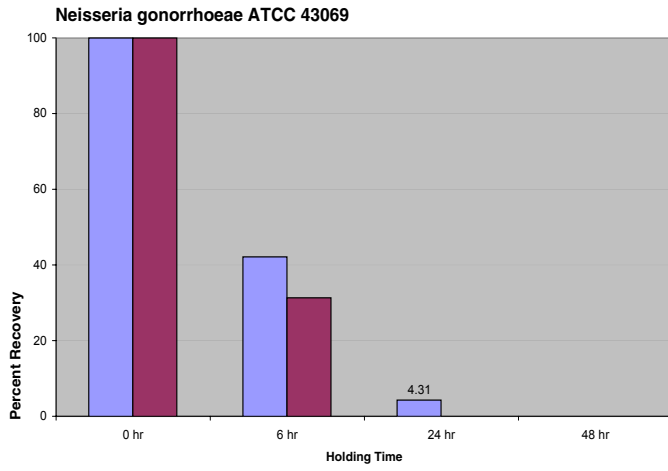
With the exception of *Bacteroides fragilis* and *Streptococcus agalactiae* and *Enterococcus* (at the 6 hour time point) BD CultureSwab Max V(+) produced higher percent recovery rates at all other time points compared to Remel BactiSwab

NCCLS M40-A standard defines acceptance criteria for swab transport system performance as follows.

1. Viability recorded at 48 hour endpoint for aerobes and anaerobes and 24 hours endpoint for fastidious bacteria (*Neisseria gonorrhoeae*)
2. No more than a 3 log₁₀ (1 x 10³ ± 10%) decline in CFU between the zero-time CFU count and the CFU of the swabs that are stored until the endpoint analysis.

Percent recovery and evaluation of swab performance against the NCCLS acceptance criteria are summarized below in Table 3.

Applying the NCCLS criteria for acceptable swab performance to our test data, BD CultureSwab Max V(+) achieved compliance with 7/8 organisms compared with 4/8 organisms with Remel BactiSwab.



DISCUSSION

Like many medical institutions our current logistics management of swab specimens involves storage or shipment of samples at ambient room temperature for between 2 – 48 hours before processing in the laboratory. For this reason we have a particular interest in how swab transport systems perform with various bacteria under these conditions. We assume that commercial swab transport systems are created equal and perform similarly however; previously published studies have demonstrated marked differences in swab performance at room temperature. In our study we evaluated two new Amies Agar Gel transport systems from Becton Dickinson and Remel and we found significant differences in their ability to maintain fastidious bacteria such as *Neisseria*, *Haemophilus* and *Peptostreptococcus*. For these three organism groups BD CultureSwab Max V(+) demonstrated higher CFU counts and percent recovery at each time point and was the only device to comply with the NCCLS M40-A room temperature performance criteria for *Neisseria* and *Haemophilus*. Less demanding organisms such as *Enterococcus*, Group B *Streptococcus* and *Bacteroides* survived well in all systems and in fact, in some cases multiplied during storage. This phenomenon is mostly likely a function of the transport/storage temperature and we intend to conduct further work comparing room temperature and refrigerated temperature transport/storage of spiked swab samples to understand better the potential advantages or disadvantages of our current specimen management practices.

Specimen collection and transportation is often overlooked or undervalued but are critical components of a Quality System for Health Care. Careful selection and evaluation of transport swab systems under the conditions and challenges these products face in routine use is important to understand product limitations and assist in improvements in specimen manage and laboratory analysis.

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Table 3 - Percent Recovery and Log₁₀ Decline

Organism / Swab Device	0 hr CFUs	0 hr % Recovery	6 hr CFUs	6 hr % Recovery	Pass/Fail	24 hr CFUs	24 hr % Recovery	Pass/Fail	48 hr CFUs	48 hr % Recovery	Pass/Fail
<i>Streptococcus agalactiae</i> (Strep B)											
BD - CultureSwab	19110	100	7167	37.5	Pass	23700	124	Pass	78890	412.8	Pass
Remel - BactiSwab	22220	100	10940	49.23	Pass	6467	29.1	Pass	1500	6.75	Pass
<i>Neisseria gonorrhoea</i> ATCC 43069											
BD - CultureSwab	5367	100	2263	42.16	Pass	231.5	4.31	Pass			
Remel - BactiSwab	1090	100	341.7	31.34	Pass	0	0	Fail			
<i>Neisseria gonorrhoea</i> clinical strain											
BD - CultureSwab	10170	100	5411	53.2	Pass	67.9	0.7	Pass			
Remel - BactiSwab	470.17	100	8.83	1.87	Pass	1.67	0.35	Pass			
<i>Haemophilus influenzae</i> ATCC 10211											
BD - CultureSwab	28590	100	23680	82.8	Pass	26280	91.9	Pass	30560	106.9	Pass
Remel - BactiSwab	22930	100	5244	22.87	Pass	23	0.1	Pass*	0	0.00%	Fail
<i>Haemophilus influenzae</i> clinical strain											
BD - CultureSwab	48560	100	28220	58.11	Pass	11640	23.97	Pass	961.7	1.98	Pass
Remel - BactiSwab	18790	100	3178	16.91	Pass	1320	7.02	Pass	4.22	0.02	Fail
<i>Enterococcus faecalis</i> clinical strain											
BD - CultureSwab	19440	100	11780	60.59	Pass	78780		Pass	231300	1189.8	Pass
Remel - BactiSwab	29780	100	28610	96.07	Pass	22180	74.48	Pass	44220	148.48	Pass
<i>Peptostrep. anaerobius</i> ATCC 27337											
BD - CultureSwab	3367	100	1034	30.7	Pass	26.22	0.78	Pass	0	0	Fail
Remel - BactiSwab	5389	100	66.67	1.24	Pass	0	0	Fail	0	0	Fail
<i>Bacteroides fragilis</i> ATCC 25285											
BD - CultureSwab	39220	100	16870	43.01	Pass	12660	32.28	Pass	1974	19.74	Pass
Remel - BactiSwab	51220	100	30220	59	Pass	23470	45.82	Pass	16870	32.9	Pass

* = borderline pass