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Does storage of sputum specimens adversely affect culture results?

F K Gould, R Freeman, S Hudson, J Magee, D Nelson, R Stafford, P R Sisson

Abstract

It has been recommended that samples submitted for microbiological examination should be retained for 48 hours after issue of the final report. In order to ascertain whether reproducible results could be achieved following storage of sputum specimens, two laboratories each re-cultured 100 samples 48 hours after their report had been issued and a further laboratory re-cultured 100 samples 48 hours after receipt. Discordant results were obtained in only 5-25% of specimens, indicating that potential respiratory pathogens could survive storage. (J Clin Pathol 1996;49:684-686)

Keywords: sputum samples storage, respiratory pathogens, microbiological examination.

working days following the issue of the final report. A third laboratory (C) retained 100 sputum specimens as above for 48 hours following receipt. All the samples were then re-cultured, usually by the same individual, but without knowledge of the previous result, according to each laboratory's SOP and the results compared. Invasive samples such as those obtained from bronchoscopy were excluded, as were specimens from patients known to be bronchiectatic in which overgrowth with *Pseudomonas* sp could be expected. The SOP for the investigation of routine sputum samples at each laboratory was briefly as follows:

LABORATORY A
Samples were refrigerated on receipt at the laboratory and processed the same day. Samples were vortexed with equal volumes of sterile deionised water until completely homogeneous and then plated onto half 5% horse blood/half heated blood agar and incubated overnight at 35°C in 5% CO₂. Plates were then returned to the incubator and re-examined after a further 24 hours' incubation.

LABORATORY B
Samples were processed on the day of receipt. Each specimen was homogenised using dithiothreitol (Sputasol, Unipath Ltd) before being cultured onto 5% horse blood, heated blood

Table 1 Comparison of results of sampling according to routine practice with sampling two to three days after the clinical report had been issued (laboratories A and B) or 48 hours after receipt (laboratory C)

	Laboratory		
	A	B	C
No of specimens yielding concordant results	79	77	95
Significant pathogen(s)	(21)	(25)	(29)
No significant pathogen(s)	(58)	(52)	(66)
No of specimens yielding discordant results	21	23	5
Total	100	100	100

Department of Microbiology, Freeman Hospital, Newcastle upon Tyne
F K Gould
D Nelson

Newcastle Public Health Laboratory, General Hospital, Newcastle upon Tyne
R Freeman
J Magee
P R Sisson

Department of Microbiology, Queen Elizabeth Hospital, Gateshead, Tyne and Wear
S Hudson
R Stafford

Correspondence to: Dr F K Gould, Microbiology Department, Freeman Hospital, High Heaton, Newcastle upon Tyne NE7 7DN.

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It has been a widely held belief amongst microbiologists that potential pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae* survive poorly in clinical specimens and so they advocate rapid processing of samples such as sputum and pleural fluid in order to maximise their diagnostic potential.¹⁻³ It was therefore surprising that the Working Party on the Retention and Storage of Pathological Records and Archives recommended that all specimens should be retained for a period of 48 hours following issue of a final report,⁴ presumably to permit confirmation of the original culture results. In order to ascertain whether reproducible results could be obtained from stored sputum samples a prospective study was coordinated in three laboratories using their individual standard operating procedures (SOPs).

Methods

One hundred consecutive sputum specimens received for routine culture and sensitivity in two diagnostic microbiology laboratories (A and B) were retained at 4°C for at least two

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Table 3 Details of bacteriological results for specimens yielding discordant results from the two samples

Results of		No. of specimens		
First sample	Second sample	Laboratory A	Laboratory B	Laboratory C
No pathogens	No growth or overgrown with non-significant bacteria	10	2	
<i>Haemophilus</i> sp	No pathogens	4	4	1
<i>S pneumoniae</i>	<i>Moraxella</i> sp	1		
<i>Moraxella</i> sp				
<i>S pneumoniae</i>	No pathogens	1	2	1
<i>S aureus</i>	Overgrown with coliform	1		
<i>Candida</i> sp	No pathogens	1		
<i>Moraxella</i> sp	No pathogens		3	
<i>Haemophilus</i> sp	<i>Haemophilus</i> sp with heavy coliform	1	1	
<i>Haemophilus</i> sp	Overgrown with other coliform		1	2
<i>Haemophilus, Moraxella</i> sp	<i>Moraxella</i> sp	1		
<i>Moraxella</i> sp	<i>Haemophilus</i> sp		1	
<i>Pseudomonas</i> sp	Overgrown with other coliform			2
No pathogens	<i>S aureus</i>		4	
No pathogens	<i>Haemophilus</i> sp		2	
No pathogens	<i>Candida</i>			3
Total		21	23	5

agar containing bacitracin and Columbia blood agar with crystal violet, nalidixic acid and gentamicin. Plates were incubated overnight at 37°C in 5% CO₂ and read the following morning.

LABORATORY C
Samples were processed on the day of receipt. Specimens were treated with an equal volume of dithiothreitol (Mucolyse, Pro-Lab), shaken for five minutes and cultured onto 7.5% horse blood with a optochin containing disc, and half blood (containing gentamicin and nalidixic acid)/half heated blood (containing bacitracin). Plates were incubated overnight at 37°C in 5% CO₂, read and then discarded.
In all three laboratories potential pathogens were identified using standard means.

Results
Table 1 summarises the results from the three laboratories. Although laboratory C achieved good correlation of results after storage of specimens for 48 hours, 21–23% of specimens handled in laboratories A and B yielded discordant results. Table 2 lists the potentially significant pathogens which were isolated from both the initial and delayed platings, revealing that from 78 (26%) of the 300 specimens a similar potential pathogen was isolated from both initial and subsequent cultures. Table 3 summarises the discordant results. Specific

pathogens failed to be isolated on second plating from 26 (8.7%) sputum samples and significant pathogens were obtained from the stored sample and not the original on nine occasions (*Staphylococcus aureus* in four samples, *H influenzae* in two and *Candida* sp in three).

Discussion
The results of this survey seem to contradict perceived wisdom that respiratory pathogens cannot survive storage,^{1 2} and show that reproducible results can be achieved in up to 75% of sputum samples which have been stored for 48 hours after the final report has been issued. However, as loss of pathogens can occur, the absence of a pathogen in a stored sample cannot exclude the possibility of its existence in the original specimen, and we would strongly recommend that all sputum samples are processed on the day of receipt.
It is interesting to note that on nine occasions a potentially significant pathogen was obtained from the stored sample and not from the original. This may be explained by the fact that not all laboratories routinely re-incubate all sputum culture plates for a further 24 hours after they have read them on the first occasion, and these pathogens sometimes require prolonged incubation.⁵
There does seem to be a difference between the laboratories in this survey in the number of discordant results, with laboratory C having only five compared with 21 and 25 in the other two. This could be explained by differences in the methodology used. Laboratory C reports all negative sputum samples after 24 hours, and thus these samples would only have to be stored for 48 hours, whereas laboratory A reports negative samples after 48 hours, thus increasing the storage time. Laboratory C re-cultured even those sputum samples yielding significant respiratory pathogens 48 hours after receipt. As it may take a further 24 or 48 hours to complete antibiotic susceptibility testing and to issue a report, similar specimens in laboratories A and B were stored for a further one to two days prior to re-culturing.
Incidentally, this survey illustrates that there are significant differences in the way in which

Table 2 Breakdown of significant pathogen(s) isolated from concordant positive samples

Pathogen(s)	No of isolates		
	Laboratory		
	A	B	C
<i>Pseudomonas</i> sp	7	1	4
<i>Haemophilus</i> sp	4	12	16
<i>S pneumoniae</i>	3	1	
<i>S aureus</i>	3	4	2
Haemolytic streptococci		1	2
Coliforms	2	4	3
<i>Haemophilus</i> sp and <i>S pneumoniae</i>			1
<i>S aureus</i> and haemolytic streptococci	1		
<i>S aureus</i> and <i>Haemophilus</i> sp		2	
Fungi	1		1
Total	21	25	29

even three neighbouring laboratories investigate routine sputum samples, and highlights the difficulties which may be faced with the potential introduction of "standardised" SOPs.

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Isolation of *Mycoplasma hominis* using the BACTEC 9000 series blood culture system

K A Kitson, K C Wright

Abstract

***Mycoplasma hominis* has been implicated as an important cause of septicaemia. There have been reported variances in the ability of blood culture systems to support the growth of this organism. In this study the ability of the BACTEC 9000 series automated system to grow and detect *M hominis* was assessed. Three of five wild *M hominis* strains grew in the BACTEC Anaerobic Plus/F medium but growth was not flagged by the detection mechanism of the system. It is recommended that users of the BACTEC 9000 series should use a seven day protocol and perform terminal subculture for suspected cases of *M hominis* septicaemia.**

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Keywords: *Mycoplasma hominis*, BACTEC 9000 series automated system.

Mycoplasma hominis may be an under-reported cause of septicaemia,^{1,2} particularly in patients who have undergone some form of genitourinary manipulation.³⁻⁵ Most frequently, the organism has been isolated from the blood of patients with postpartum pyrexia.² There may also be an increased incidence in immunosuppressed patients³ and in neonates.⁴

Previous studies have shown the failure of some blood culture media to support the growth of *M hominis*.^{1,2,4,6} In many cases this failure has been attributed to the presence of sodium polyanethyl sulphonate (SPS), a substance to which mycoplasma and some other bacteria are known to be susceptible.^{2,5}

The Becton Dickinson (Cowley, Oxford, UK) BACTEC 9000 automated system uses culture media that include 0.05% SPS in their formulation along with a mixture of resins intended to neutralise the effects of antibiotics in the patient's blood. A fluorescence sensor incorporated into the bottle detects carbon dioxide production as an indicator of microbial growth. Positive cultures are flagged when the

appropriate changes in the continuously monitored samples are detected by the indicator system.

It is recommended by Becton Dickinson that to counteract any inhibitory effect of SPS and to optimise the isolation of susceptible organisms 10 ml of blood should be added to their Plus/F blood culture media.

The aim of this study was to assess the ability of the medium used in the BACTEC 9000 series system to support the growth of *M hominis* and to assess the effectiveness of the system for detecting this organism from blood culture.

Methods

The methods used were similar to those used in a study by Davies and Spencer.¹ Five strains of *M hominis* were isolated from female genital specimens. Colonies were subcultured for purity onto Columbia blood agar (Becton Dickinson) and identified by morphology, resistance to erythromycin, ability to utilise arginine, and inability to ferment glucose or hydrolyse urea.² Cultures were incubated in anaerobic jars using Anaerogen sachets (Oxoid, Basingstoke, UK).

A 1 cm² block of Columbia blood agar with pure confluent growth of *M hominis* was removed from the plate. This block was agitated in 10 ml sterile isotonic saline, and 1 ml of the supernatant was removed and diluted in another 9 ml sterile saline. Finally, 1 ml of this dilution was pipetted into 99 ml sterile, defibrinated horse blood. Surface viable counts were performed on all dilutions to ascertain the inoculum size.

Duplicate BACTEC Aerobic Plus/F and Anaerobic Plus/F bottles were inoculated with 10 ml of the prepared blood. One pair from each set remained in the machine undisturbed while the other was removed daily for subculture. Brain heart infusion (BHI) broth was inoculated as a viability control (Technical Service Consultants, Heywood, Lancs, UK).

Daily subcultures were carried out for seven days on the control bottles and one of the sets

Department of Microbiology, Huddersfield Royal Infirmary

Correspondence to: Mr K A Kitson, Chief Biomedical Scientist, Department of Microbiology, Huddersfield Royal Infirmary, Lindley, Huddersfield HD3 3EA.

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