

Gas-liquid chromatography in the diagnosis of anaerobic infections: a three year experience

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SUMMARY Nearly two thousand clinical samples were examined by direct gas-liquid chromatography over a three year period. Absence of volatile fatty acids (VFAs) in the samples correlated well with negative culture results for anaerobic bacteria. In general the presence of acetic acid alone correlated well with the presence of aerobic organisms, whereas the presence of a mixture of VFAs correlated well with the presence of anaerobic organisms, either alone or in combination with aerobes. However a proportion of such VFA-positive samples gave no growth on culture. Swabs gave comparable results to samples of pus or exudates except that a higher proportion of the former were VFA-negative but culture positive.

Gas-liquid chromatography (GLC) is now a well established technique for bacterial characterisation. Since the uses of the technique were reviewed in 1975,¹ gas-liquid chromatography has also been developed as a method for the rapid diagnosis of anaerobic infection.²⁻⁴

Although the technique is now well established, there has been little critical appraisal of its usefulness in the diagnostic laboratory as opposed to the research laboratory. Such an appraisal is necessary in view of the considerable capital and recurrent costs associated with the technique. The present paper records our general experience of direct gas-liquid chromatography in a busy diagnostic laboratory over a three-year period; a critical evaluation of the technique in the light of these results is presented in a separate paper.⁵

Material and methods

SAMPLES

The range of samples processed are shown in Table 1. The samples were not selected in any way—they were not restricted to those thought likely to yield anaerobic bacteria on culture. “Intestinal secretions” included samples of ileostomy fluid, nasogastric aspirate and gastric washings; “other fluids” included peritoneal fluid, ascitic fluid, bursa fluid, hydrocoele fluid, semen, amniotic fluid, cyst fluid, sinus washings, lymph and sub-dural effusion. “Mis-

cellaneous” samples included biopsy material, sputum, marrow, uterine curettings, bone sealant, vomit and faeces.

Transport and receipt of specimens

In general, specimens were received in the laboratory 2–3 h after collection.

Bacterial isolation

All specimens were plated out on conventional media for aerobic and anaerobic incubation. In addition, each sample was seeded on to a plate of menadione blood agar (see below) with a 5 µg metronidazole disc placed in the “well” and the plate incubated anaerobically,⁶ for at least 48 h without

Table 1 *Specimens examined by direct gas-liquid chromatography*

Type of sample	No examined	No (%) negative*	No (%) positive†
Pus	251	112 (44.6)	139 (55.4)
Pleural aspirate	123	114 (92.7)	9 (7.3)
Joint aspirate	232	225 (97.0)	7 (3.0)
Intestinal secretions	56	29 (51.8)	27 (48.2)
Drain fluids	319	233 (73.0)	86 (27.0)
Wound aspirates	362	304 (84.0)	58 (16.0)
Bile	103	78 (75.7)	25 (24.3)
Other fluids	191	163 (85.3)	28 (14.7)
Miscellaneous	32	12 (37.5)	20 (62.5)
Swabs	128	92 (71.9)	36 (28.1)
Blood cultures	132	104 (78.8)	28 (21.2)
Total	1929	1466 (76.0)	463 (24.0)

*No volatile fatty acids detected.

†One or more volatile fatty acids detected.

disturbance. Aerobic isolates were identified according to Cowan,⁷ and by the API 20E system (API Laboratory Products Ltd, Basingstoke) where appropriate. Anaerobic isolates were identified as previously described.⁸

Menadione/haemin blood agar

Columbia blood agar base (Oxoid) was supplemented by 10% horse blood and by haemin and menadione to final concentrations of 5 mg/l and 1 mg/l respectively.

GAS-LIQUID CHROMATOGRAPHY

Extraction of volatile fatty acids

A portion of the sample (1 ml) was acidified with five or six drops of 50% sulphuric acid in a glass tube then shaken to mix. Ether (1 ml) was added, the mixture was shaken vigorously then centrifuged at 2500 rpm for 30–60 s. The ether layer was then carefully withdrawn and 1 ml of this was injected into the column.

Extraction of non-volatile fatty acids

Sample (or culture supernatant) (1 ml) was added to 0.5 ml 50% H₂SO₄ in a glass tube and mixed by shaking. Methanol (2 ml) (methanol for chromatography, BDH) was added and the mixture incubated at 56°C for 30 min. After cooling to room temperature, 1 ml of chloroform was added, the tube was inverted four or five times to mix and was then centrifuged at 2500 rpm for 30–60 s. The chloroform layer was carefully removed and 1 ml of this was injected into the column.

Extraction of volatile fatty acids from swabs

Each swab was placed in a glass tube containing 1 ml of sterile distilled water and briskly rotated 20 times, then squeezed against the side of the tube to remove as much fluid as possible and then withdrawn. The tube was then shaken and the fluid treated as indicated for pus or exudates (see above).

Columns

Glass columns 1.5 mm × 4 mm internal diameter were used, packed with 15% SP-1220 1% H₃PO₄ on 100/120 Chromasorb WAW 1-2144 (Supelco, USA). In the latter part of the studies, supplies of this material were no longer available and after a series of trials of other materials, 2Q/10% FFAP (Pye Unicam, Cambridge, England) was used as an alternative.

Conditions for chromatography

Chromatography was performed on a Pye Unicam 204 chromatograph fitted with a flame ionisation

detector. In early studies samples were injected manually; in the latter part of the study, an automatic sampling device (Autojector, Pye Unicam) was fitted. The conditions used for chromatography were as follows:

Carrier gas (oxygen-free nitrogen)	35–40 ml/min
Hydrogen	80–85 ml/min
Air	215–220 ml/min
Oven temperatures as follows:	detector 250°C
	column 160°C
	injector 125°C

Attenuation 4×10^2 .

Detection of non-volatile acids in clinical samples

It was suggested to us (FP Tally, personal communication 1979) that the presence of succinic acid in clinical samples was a more reliable indicator of anaerobic infection than the presence of VFAs. In a preliminary study of 60 samples, we found no correlation between the presence or absence of this acid and the presence of anaerobic bacteria in the sample. We therefore did not continue to look for non-volatile fatty acids in subsequent samples.

Detection of fatty acids

The chromatographs were produced as paper traces on a flat bed recorder (Belmont Instruments Ltd). Fatty acids were identified by comparison of the retention times of peaks in the test samples (clinical samples or culture filtrates) with those of known standard solutions. The concentration of fatty acids in a sample was assessed semiquantitatively by comparison of the peak heights of the test sample with those of the corresponding fatty acids in the standard solution. Concentrations of a given acid that were less than 10% of that of the corresponding standard were ignored.

Results

A total of 1929 samples was examined (see Table 1). The 32 miscellaneous samples included biopsies, sputum, bone marrow, uterine curettings, vomit, faeces, and a single sample of bone sealant. Of the 1929 samples, 1466 (76.0%) yielded no volatile fatty acids (VFAs); 463 (24.0%) were GLC-positive—that is, yielded one or more VFAs on gas-liquid chromatography. The percentage of VFA-positive samples varied for different categories of sample (see Table 1). For example, 55.4% of pus samples were VFA-positive as compared with 3.0% of joint aspirates.

The cultural results for the VFA-negative samples (Table 2) showed that an appreciable number yielded an aerobic growth on culture but only 21

Table 2 Culture results from VFA-negative* samples

Type of sample	Total No VFA-neg*	No (%) negative on culture	No (%) yielding aerobic growth only	No (%) yielding anaerobic growth only
Pus	112	53 (47.3)	57 (50.9)	2 (1.8)
Pleural aspirate	114	107 (93.9)	7 (7.9)	—
Joint aspirate	225	209 (92.9)	16 (7.1)	—
Intestinal secretions	29	15 (51.7)	12 (41.4)	2 (6.9)
Drain fluids	233	184 (79.0)	46 (19.7)	3 (1.3)
Wound aspirates	304	233 (76.6)	71 (23.4)	—
Bile	78	45 (57.7)	32 (41.0)	1 (1.3)
Other fluids	163	123 (75.5)	35 (21.5)	5 (3.0)
Miscellaneous	12	8 (66.7)	4 (33.3)	—
Swabs	92	34 (37.0)	50 (54.3)	8 (8.7)
Blood cultures	104	92 (88.5)	12 (11.5)	—
Total	1466	1103 (75.2)	342 (23.3)	21 (1.4)

*No volatile fatty acids (VFAs) detected on gas-liquid chromatography.

Table 3 Bacterial isolates from VFA-negative fluids yielding anaerobic bacteria on culture

Type of sample	Anaerobic isolates	Aerobic isolates
Pus	<i>B fragilis</i>	<i>E coli</i> ; <i>Proteus</i> sp
Pus	<i>C perfringens</i> (<i>C welchii</i>)	—
Paracentesis fluid	<i>B fragilis</i>	—
Semen	Anaerobic cocci	—
Semen	<i>B fragilis</i>	<i>Staph albus</i>
Bile	<i>C perfringens</i> (<i>C welchii</i>)	<i>E coli</i>
Cyst fluid (pancreas)	<i>C perfringens</i> (<i>C welchii</i>)	—
Liquor amnii	<i>B fragilis</i>	—
Nasogastric aspirate	<i>Bacteroides</i> sp	—
Drain fluid	<i>B fragilis</i>	—
Paracentesis fluid	<i>B fragilis</i>	—
Drain fluid	<i>B thetaiotaomicron</i>	<i>E coli</i>
	<i>B uniformis</i>	<i>Proteus</i> sp
	Anaerobic cocci	—
	<i>C perfringens</i>	—
Drain fluid	<i>B fragilis</i>	—

(1.4% of the VFA-negative samples) yielded anaerobes on culture (false-negative samples). Of these, five were other fluids and eight were swabs. Only two samples of pus gave false-negative results. The organisms isolated from the samples yielding aerobic growth but no VFAs on GLC included a wide variety of different species. The commonest organisms were *Staphylococcus aureus* (74 isolates), *Staph albus* (45 isolates), *Escherichia coli* (33 isolates), with smaller numbers of isolates of streptococci, *Haemophilus* spp, and other enterobacteria. *Candida albicans* was isolated from 12 samples. A mixed aerobic flora was present in 31 samples.

Of the 32 samples of bile yielding aerobic growth, 16 samples yielded *E coli* alone or in combination, the remainder yielded a variety of bacterial species including enterococci, *Staph aureus* and one isolate of yeasts.

Of the 16 joint aspirates showing growth, most yielded *Staph aureus* although one yielded a growth of *Neisseria gonorrhoeae*. The anaerobes isolated from the VFA-negative fluids are shown in Table 3

and the results for the swabs discussed below.

In the case of the VFA-positive samples (Table 4), anaerobes were isolated (alone or in combination with aerobes) from 203 of the 469 VFA-positive samples (43.8%). This represents 10.5% of the total number of samples processed. Anaerobes alone were isolated from 97 (20.9%) of VFA-positive samples. A total of 42 VFA-positive samples gave no growth on culture. Almost all of these gave acetic acid as the sole VFA; only four samples gave VFA patterns with four or more VFAs and of these two were from patients on concurrent metronidazole therapy.

SWABS

A total of 128 swabs was examined. Of these, 92 were VFA-negative and 36 VFA-positive. Of the VFA-negative swabs, 34 (37.0%) were negative on culture but 50 (54.3%) yielded a growth of aerobes and eight (8.7%) yielded a mixed growth of aerobes and anaerobes. Of the VFA-positive swabs, only two (5.5%) failed to give any growth on culture; 14

Table 4 Culture results from VFA-positive* samples

Type of sample	Total No VFA-pos*	No (%) negative on culture	No (%) yielding aerobes	No (%) yielding anaerobes	No (%) yielding aerobes + anaerobes
Pus	139	5 (3.6)	61 (43.9)	38 (27.3)	35 (25.2)
Pleural aspirate	9	4 (44.4)	—	3 (33.3)	2 (22.3)
Joint aspirate	7	3 (42.8)	1 (14.3)	2 (28.6)	1 (14.3)
Intestinal secretions	27	1 (3.7)	11 (40.7)	6 (22.2)	9 (33.3)
Drain fluids	86	5 (5.8)	42 (48.8)	13 (15.1)	26 (30.3)
Wound aspirates	58	3 (5.2)	40 (69.0)	11 (18.9)	4 (6.9)
Bile	25	6 (24.0)	12 (48.0)	4 (16.0)	3 (12.0)
Other fluids	28	5 (17.9)	8 (28.6)	9 (32.1)	6 (21.4)
Miscellaneous	20	—	13 (65.0)	1 (5.0)	6 (30.0)
Swabs	36	2 (5.5)	14 (38.9)	6 (16.7)	14 (38.9)
Blood cultures	28	8 (28.6)	9 (32.1)	11 (39.3)	—
Total	463	42 (9.1)	211 (45.6)	104 (22.5)	106 (22.9)

*One or more volatile fatty acids detected on gas-liquid chromatography.

Table 5 Principal VFA patterns and cultural results

VFA pattern	No of samples yielding aerobic growth only	No of samples yielding anaerobic growth only	No of samples yielding aerobic and anaerobic growth
A	139	9	4
B	—	2	3
A, P	22	4	17
A, i-B	1	1	1
A, B	10	10	4
A, i-V	12	—	1
A, P, B	9	2	14
A, P, i-V	—	—	2
A, i-B, i-V	3	4	1
A, i-B, V	—	—	1
≥ 4 VFAs present	22	67	62
Total	218	99	110

A = acetic acid.
 B = butyric acid.
 P = propionic acid.
 i-B = isobutyric acid.
 i-V = isovaleric acid.
 V = valeric acid.

(38.9%) yielded a growth of aerobes, six (16.7%) a growth of anaerobes and 14 (38.9%) yielded a mixed growth of aerobes and anaerobes.

BLOOD CULTURES

A total of 132 blood cultures were examined, of which 104 (78.8%) were VFA-negative. None of these yielded anaerobic bacteria on culture. Of the VFA-positive cultures, eight (all showing acetic acid only) failed to yield any bacterial growth on culture. Of the remainder four yielded anaerobes (all with three or more VFAs present) and 16 aerobes. Of the latter samples, 13 gave acetic acid only, two acetic and propionic acid, and one (subsequently yielding *Propionibacterium acnes* on culture) propionic, acetic and butyric acids.

VFA PATTERNS

The principal VFA patterns obtained are shown in Table 5. In general, the presence of acetic acid alone was most commonly associated with aerobic growth

and the presence of a complex VFA pattern (four or more VFAs present) with anaerobic or mixed aerobic and anaerobic growth. A more detailed analysis of the VFA patterns is given elsewhere.⁹

Discussion

The use of direct gas-liquid chromatography as a procedure for the rapid diagnosis of suspected anaerobic infection has been reported on by several workers,^{2,3,10-12} but there have been few reports of its use as a routine procedure for unselected samples in the diagnostic laboratory. In the present study, our results show that for such unselected samples (that is, samples examined routinely without selection as to their likelihood of yielding anaerobic bacteria) more than three-quarters were negative on gas-liquid chromatography, with only a very small proportion of these being false-negative—subsequently yielding anaerobic bacteria on culture. Thus a negative result on gas-liquid chro-

matography—that is, no VFAs present, is a very good presumptive indication that no recoverable anaerobic bacteria are present in a sample. It is clear however that nearly a quarter of such negative samples will subsequently yield aerobic bacteria on culture.

Of those samples yielding one or more VFAs—that is, positive on gas-liquid chromatography, nearly 10% gave no growth on culture. At least some of these samples may have been from patients on concurrent antimicrobial therapy (possibly including metronidazole) but we were unable to obtain accurate information on this point. Results of a further study in which we sought such information are presented elsewhere.⁵ It is interesting that some types of sample, notably bile, yielded a much higher proportion of VFA-positive, culture-negative specimens than the average. The reasons for this finding are not clear, but may reflect the fact that a majority of patients undergoing biliary surgery receive perioperative antimicrobial chemotherapy.

Nearly half of the VFA-positive samples yielded aerobic growth only, associated in the main with the presence of only one or two VFAs, whereas the samples yielding anaerobic bacteria on culture gave in general a more complex VFA pattern. A more detailed analysis of the VFA patterns obtained is given elsewhere, but it is clear that in general, the presence of acetic acid only suggests aerobic infection (but note that a considerable proportion of samples yielding aerobic growth only showed *no* VFA), whereas the presence of a complex VFA pattern is more commonly associated with the presence of anaerobes in the sample. These correlations are not absolute—10% of samples yielding aerobic growth had four or more VFAs present. This may indicate inability to recover anaerobic bacteria from the sample, possibly because of concurrent antibiotic therapy.

Some workers have suggested that extraction of VFAs from swabs may be of value in indicating the presence of anaerobic bacteria.¹³ In our experience, clinicians commonly send swabs instead of samples of pus or exudates, even though the survival of anaerobic bacteria on swabs may be poor,¹⁴ with organisms being retained in the swab and not recovered on culture. In the present study, only two of the VFA-positive swabs failed to yield organisms on culture, whereas 10% of VFA-negative swabs did in fact yield anaerobes on culture. Thus, if pus or exudates are not available for direct gas-liquid chromatography, swabs may be an acceptable alternative.

In the present study, we have investigated the role of direct GLC in the diagnosis of anaerobic infections; we were not able to repeat the GLC examina-

tion in samples from patients undergoing therapy for such infections. It has been suggested that direct chromatography could be used to monitor therapy in anaerobic infections;¹⁵ we hope to investigate this possible role of the technique in future studies.

The present paper has outlined the results of a three-year experience of direct gas-liquid chromatography. This confirms that in general gas-liquid chromatography (GLC) is a useful screening test for the presence or absence of anaerobic infection, but not of aerobic infections. The following questions remain to be answered:

Can GLC provide an indication of the type of anaerobic organisms present in a sample?

Can GLC provide any useful information on the presence or absence of specific types of aerobic organisms—for example, *Staph aureus*?

Is there a correlation between the VFA patterns from clinical samples and the VFA patterns of anaerobic isolates recovered from such samples?

These questions are considered in the accompanying paper.⁹

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