Isolation rates of *C difficile* in five groups of subjects

Group	No of subjects	Description of subjects	Specimen	Media	Isolation rate
I	62	Consecutive women attending DGM*	Single HVS	CCF agar	0/82
II	90	Consecutive women attending DGM*	Single HVS	CCF agar RCA + 0.1% <i>p</i> -cresol	1/50 0/50
III	82	Consecutive women attending DGM*	Duplicate HVS	CCF agar	1/82 (1.2%) $p < 0.01$
IV (a)	90	Mothers predelivery	Duplicate HVS	CCF broth	9/82 (11%)
IV (b)	45	Mothers postdelivery	Duplicate HVS	CCG agar	0/50 (0%) $p < 0.0027$
V	50	Neonates 2nd-5th day	Swab from rectum or soiled nappy	CCF broth	9/50 (18%) 2/45 (4.4%) 4/45 (8.8%) 29/50 (58%) 28/50 (56%)

*Department of Genital Medicine.

p was derived from standard error (SE) using SE of differences in proportion = $\sqrt{\frac{p(100-p)}{n1} + \frac{p(100-p)}{n2}}$

HVS = high vaginal swab.

NEONATAL STOOL SPECIMENS (GROUP V)

In contrast to the results obtained with vaginal swabs, the isolation rate obtained using CCFT broth was not significantly different to that obtained using CCF agar. This may be explained by the relatively high counts of *C difficile* organisms present in many faecal specimens and by the occasional failure of CCFT broth to yield *C difficile* when overgrowth of "coliform" organisms had taken place in the broth.

In conclusion we suggest that a liquid culture medium, such as that described here, is a useful addition to conventional agar culture when only small numbers of *C difficile* organisms are likely to be present.

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Rapid demonstration of nucleic acids using "oxidised" gallocyanin and chromic potassium sulphate: methods and applications

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Studies of metallic salt lakes of the oxazine dyes gallamin blue, celestine blue and gallocyanin led to the introduction of techniques for selective staining of Nissl and nuclear substance in nerve cells¹ and specific nuclear stains²⁻⁴ and to the theory of gal-

locyanin chromalum staining and its application for quantitative estimation of basophilia.⁵

The gallocyanin chromalum technique of Einarsson¹ was reviewed by us for use with the Quantimet image analysing computer⁶ and has now been adopted as a nuclear stain for the automated interactive cervical cancer screening system CERVIFIP where detection by integrated optical density is utilised, but its use in routine service conditions is limited by the fact that the staining technique requires incubation at 42°C for 16 h.⁷

We have therefore conducted experiments to reduce the staining time required to a minimum but still retain its degree of stoichiometry and stain density. Our results have also shown that the rapid staining technique is useful for staining cells other than those exfoliated from the cervix, namely

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squamous cells from the buccal cavity in the demonstration of Barr bodies and for the staining of spermatozoa or cells from other body system sites where a degree of stoichiometry or pattern recognition is required.

Material and methods

Aqueous solutions of gallocyanin "Gurr" (BDH Chemicals) with chromic potassium sulphate Analar (BDH Chemicals) were prepared. The concentrations of gallocyanin were varied from 0.25 g% to 2.5 g% in 0.25 g intervals. Two aliquots for each weight value were prepared. *Schedule 1* in which gallocyanin was reacted with concentrated H_2SO_4 (1 ml concentrated H_2SO_4 per 0.5 g of gallocyanin) and *Schedule 2* where the smears were stained using "unoxidised" gallocyanin. The chromic potassium sulphate was titrated by varying the concentration from 2.5 g% to 25.0 g% in 2.5 g intervals. The chromic potassium sulphate was prepared by dissolving in distilled water and gently warming on a hot plate and the gallocyanin added accordingly to the schedule. The solutions were then boiled for 5 min, allowed to cool, filtered and the original volume restored by adding distilled water. The pH was then measured and set at 1.0.

Cervical material collected from women attending family planning, gynaecological out patient and colposcopy clinics, using poly-L-lysine coated slides, was used to prepare monolayers^{8,9} which were stained in the solutions at 20°C for 5 min. The results of staining by both schedules were assessed visually and scored according to the degree of specificity and stain intensity. Two different combinations of gallocyanin were selected from those which demonstrated a high degree of specificity and sufficient stain intensity, namely 0.5 g% and 1.0 g% "oxidised" gallocyanin with 17.5 g% chromic potassium sulphate.

These two combinations were then used to stain cervical monolayers prepared by pooling material collected from 20 women attending antenatal and colposcopy clinics. The staining was carried out at 20°C and pH 1.0 but slides from the pooled samples were stained by both methods for 2, 5, 10, 15 and 20 min. The Einarson' 18 hour-gallocyanin chromalum technique was included as a control.

The staining results were assessed using a Vickers M85 integrating microdensitometer by measuring the integrating optical densities of 20 nuclei selected at random. Absorption maxima were determined for the Einarson 18-hour schedule as well as the new 2-minute schedule at concentrations of 0.5 g% and 1.0 g% "oxidised" gallocyanin with 17.5 g% chromic potassium sulphate (Table 1). The microdensitome-

ter was set at a wavelength of 550 nm it being the optimum for the gallocyanin stain used, and all measurements were made with a $\times 40$ objective, scanning spot size 0.5 μm and closing mask of 20 μm diameter.

Results

VISUAL ASSESSMENT OF GALLOCYANIN CHROMIC POTASSIUM SULPHATE STAINED SLIDES

The specificity of nuclear staining was acceptable in solutions where the chromic potassium sulphate concentration was >10.0 g%. However, staining intensity appeared to improve at higher concentrations of gallocyanin (>0.25 g%) particularly after prior "oxidation" with concentrated H_2SO_4 .

DATA FROM NUCLEAR MEASUREMENTS

The mean, standard deviation and standard error of the mean of the relative absorption of 20 nuclei selected at random on each slide are shown in Table 2. All of the values of relative absorption for the rapid staining schedules were greater than that of the control gallocyanin (43.2). The difference in relative absorption (ie stain density) between the increasing incubation times was relatively small. In fact, adequate nuclear staining was achieved within two minutes. Some cytoplasmic absorption of the dye solution was noted in cells incubated in stain for more than 5 min.

Discussion

The results of this preliminary study have shown that higher concentrations of chromic potassium sulphate lakes of "oxidised" gallocyanin increase the rate of staining reaction and have effectively produced a rapid and simplified method for the demonstration of basophilic cell structures namely nucleic acids. Not only has this technique resolved a problem in the preparation of cervical material for automated screening and in assessing the DNA changes in other cytological preparations; it is also a suitable and useful method which has applications in the fields of forensic pathology and cytogenetics for the detection of spermatozoa and the demonstration of Barr bodies.

A more elaborate study is being pursued together with our associates, Dr J Tucker, M Stark and P Eason of the MRC Clinical and Populations Cytogenetic Unit, Edinburgh and will be the subject of a further communication.

Table 1 Determination of absorption maxima for the 18 hour Einarson gallocyanin-chromalum (GCA) and the rapid two minute 0.5 g% and 1.0 g% techniques

Wavelength (nm)	400	410	420	430	440	450	460	470	495	510	530	550	570	620	650
18 hour GCA	15	23	26	28	31	31	32	33	37	38	42	43	43	37	28
0.5 g% 2 min	11	12	12	14	14	16	18	22	27	36	43	48	46	43	32
1.0 g% 2 min	8	8	15	15	16	21	25	29	38	48	56	64	54	58	46

Absorption maxima is 550 nm for all three techniques.

Table 2 Relative absorption of 20 nuclei read at 550 nm

	18 hour control	0.5 g% oxidised gallocyanin 17.5 g% chromic potassium sulphate					1.0 g% oxidised gallocyanin 17.5 g% chromic potassium sulphate				
		Minutes					Minutes				
		2	5	10	15	20	2	5	10	15	20
Mean	43.2	53.35	57.55	64.0	66.15	65.0	63.2	65.19	68.25	75.2	88.2
SD	6.42	5.99	8.12	9.07	6.86	6.67	5.74	8.02	8.21	15.45	8.86
SEM	1.43	1.43	1.81	2.02	1.53	1.49	1.28	1.75	1.83	3.45	1.98

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Letters to the Editor

A fluorescent technique for demonstrating the chromatoid bodies and nuclei in cysts of *Entamoeba histolytica* from faecal deposits

The diagnosis of *Entamoeba histolytica* cysts depends on the observed criteria of size, number of nuclei and presence of chromatoid bodies (chromidial bar) which have blunt rounded ends. Whilst all of these may be clearly seen in freshly prepared deposits of faeces, samples of several days old may contain granular cysts, making the diagnosis more difficult.

Acridine orange can be used to stain the relevant DNA/RNA components of a cyst which when viewed under UV light show the chromidial bars as bright orange or

green structures, and stain the nuclei green.

This staining technique represents a useful alternative to Sargeant's stain,¹ as the cysts and their chromidial bars are readily visible on scanning the preparation under low power. This technique requires only 30 min staining time, the other staining techniques require the material to be stained for 24 h or longer for ideal results. It also give satisfactory results on saline suspension of unconcentrated faeces, unlike the method of Sargeant.

The staining method is a modified version of that of Bertalanffy and Bickis,² and is prepared as follows:

Stock solution: 0.1% acridine orange in distilled water.

For use: 1 ml stock acridine orange solution

0.5 ml acetic acid

8.5 ml Krebs-Ringer solution

This provides a working solution of pH below 4 (The actual pH is not critical providing it is below pH 4.5).

Technique: Faeces may be fresh suspension or concentrated by the standard formol-ether technique.³

An equal volume (approx two drops) of working acridine orange stain is added to the deposit and mixed well.

After 30 min or longer at room temperature a drop of the deposit is placed on a slide with a coverslip and examined under a mercury vapour UV light source using a BG12 filter or an iodine quartz epifluorescent UV source, using a high power dark ground condenser to provide a black background.

The cysts are seen staining pale green with the chromidial bars staining bright orange or green and the nuclei stain green, against a black background. (No loss of