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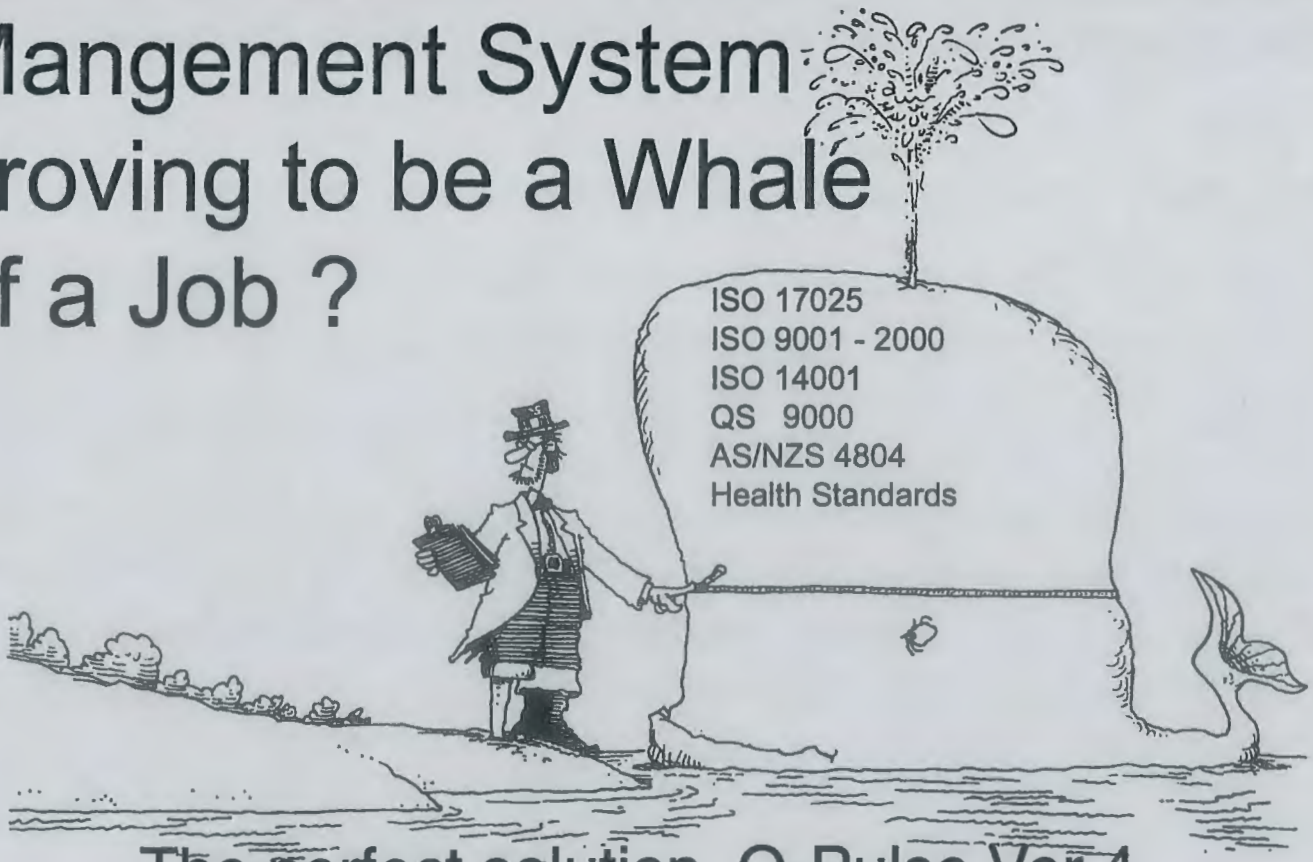

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Asthma in medical laboratory workers

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Occupational asthma describes asthma caused by a specific workplace exposure and is not due to factors outside the workplace. It needs to be distinguished from asthma which is coincidental to the workplace but which may be aggravated by irritant exposures at work (similar to the aggravation which may occur in most asthmatics on exposure to second-hand smoke, paint fumes, dusts or cold air). Occupational asthma is most commonly due to an immunologic or presumed immunologic response to a workplace sensitiser. This may be an IgE mediated response to a high molecular weight allergen (often a protein), or to certain chemicals such as epoxy resins. However, some workplace sensitisers may cause a clinical response similar to this but without a clearly identified IgE-mediated response. Less commonly (about 6% of cases of occupational asthma), the mechanism may be a single very high level irritant airway exposure, resulting in Reactive Airway Dysfunction Syndrome (RADS), or Irritant-Induced Asthma (1,2).

Medical laboratory causes of occupational asthma

There are many laboratory settings in which occupational asthma may occur. In most laboratory settings workers wear natural rubber latex gloves for protection and/or maintaining sterility. Especially in atopic workers, the use of high protein powdered natural rubber latex gloves will expose the worker to a risk of IgE-mediated sensitisation to the gloves and subsequent asthma from natural rubber latex, carried in the glove powder. The prevalence of natural rubber latex induced occupational asthma in healthcare workers has been reported to be 5-17% (3). Among 52 clinically sensitised hospital workers from our centre identified up until 1999, with positive skin prick tests to natural rubber latex, 6 were medical laboratory workers (12%). We previously reported airborne natural rubber latex levels of over 300 nanograms per cubic litre of air in our haematology laboratory during routine usage of powdered natural rubber latex gloves, with a marked reduction after changes to powder-free, lower protein natural rubber latex gloves (4). Rates of new sensitisation to natural rubber latex gloves in our hospital staff also markedly fell with the change in glove type to low-protein, powder-free latex gloves (5).

Other high-molecular weight sensitisers to which medical laboratory staff may have exposure include rats, mice and other laboratory animals (in research laboratories), and enzymes (e.g. papain in blood-banks, and other enzymes used for biochemical reactions in the laboratory setting).

Low-molecular weight sensitisers include glutaraldehyde (e.g. used to sterilise equipment in pulmonary function and other laboratories), and medications such as penicillins which may be mixed by pharmacy technicians. Radiation technologists also may have exposure to diisocyanates e.g. methylene diphenyl diisocyanate has been used in radiotherapy settings to custom-produce moulds to keep patients in the correct position for treatment.

Other occupational sensitisers (e.g. quatternary ammonium compounds or pine products) may also be present in cleaning agents used by laboratory workers or the house-keeping staff in the laboratories, although cleaning agents also are often respiratory irritants and it may be difficult in all cases to characterise a response as being due to a sensitiser.

Irritant-Induced Asthma or RADS may be induced if the medical laboratory worker has an exposure to a very high level of a respiratory irritant, such as a spill of acetic acid (as reported in a hospital setting by Kern and colleagues) (6), or a fire in the laboratory. More commonly, asthma which starts coincidentally to the workplace may be aggravated by exposure to respiratory irritants in the medical laboratory, such as strong cleaning agents e.g. bleach or other agents, acids, or ammonia (as when cleaning animal cages). Endotoxin exposure may occur from animal feed or animal cages, and this can aggravate asthma but also in animal models may act as an adjuvant augmenting sensitisation to natural rubber latex (7).

Investigation of workers with suspected occupational asthma

Investigation of these workers should be performed as for other patients with suspected occupational asthma, according to published guidelines and consensus statements (8,9). The diagnosis should be suspected in any worker with new-onset asthma, especially if there is worsening of symptoms at work and/or improvement on weekends or holidays off work.

An objective diagnosis, of asthma should initially be confirmed by pulmonary function testing and/or methacholine challenge when the patient has been at work. The occupational component to this can be assessed by evidence of an improvement in serial peak flow readings, serial spirometry and/or methacholine or histamine challenge responses off work as compared with results while working. Examination of eosinophil counts in induced sputum during work periods and times off work may add to the diagnostic certainty although assessing exhaled nitric oxide levels has not to date been shown to be of value (10). The causative agent may be suspected from the history of exposure and review of material safety data sheets from the work area, but new sensitising agents are reported each year, so the absence of a recognised sensitiser on the material safety data sheets does not exclude this possibility. Unfortunately there are few commercial skin test extracts available for high-molecular weight work-place sensitisers, and reliable in-vitro immunological tests for these agents are limited to a few centres. Specific challenge tests either in the workplace setting or in a controlled environmental exposure chamber can also assist in the diagnosis and in identifying the causative sensitiser.

Irritant-Induced Asthma remains a circumstantial diagnosis, relying largely on the exposure history and objective evidence of asthma, with no previously documented respiratory disease.

Management

As with occupational asthma in other settings, medical laboratory workers who have occupational asthma related to a workplace sensitiser should be completely removed from further exposure to that sensitiser. If occupational asthma is due to natural rubber latex, then a change to powder-free, low-protein natural rubber latex glove use by co-workers and personal use of non-natural rubber latex gloves may be sufficient to prevent further work-related symptoms. In the case of other causative sensitisers, it may be possible to change the laboratory agent to a different agent, but in many cases, the worker can only

completely avoid exposure by a move to a different laboratory. The outcome of occupational asthma has been shown to relate to the duration of exposure to the work sensitiser after the onset of asthma, and to the severity of asthma at the time of stopping exposure. The best prognosis is with early diagnosis and removal from further exposure.

Racal-type air supply respirators may provide short-term protection for workers with occupational asthma who need occasional exposure to the responsible sensitiser, such as natural rubber latex or animals (10). However, such use has not been shown to be uniformly effective for long periods of work, and avoidance of work areas containing airborne levels of the sensitiser remain preferable.

As with sensitiser-induced occupational asthma in other settings, the patient's asthma should be managed by control of exposure to other relevant allergens and respiratory irritants as well as with appropriate medications. Workers may be eligible for workers' compensation (in Canada), and consideration should be given to screening of other exposed workers for occupational asthma. Occupational hygiene review of the laboratory environment may assist in reducing exposure for other workers to respiratory sensitisers.

Workers with Irritant-Induced Asthma, can usually continue to work in the same environment with appropriate medical treatment of their asthma, as can workers with workplace aggravation of coincidental asthma.

Prevention of occupational asthma in the workplace

For many occupational sensitisers it has been shown that the risk of occupational asthma in a workplace increases with increasing airborne exposure levels. As reviewed by Bush and colleagues (11), laboratory animal handlers (who clean the cages, feed and care for the animals) are at much greater risk of sensitisation than those who use the animals for experimental purposes (such as technicians, students and investigators). At least risk are relatively unexposed workers in the area such as secretaries and administrators.

Therefore, maintaining airborne levels of work sensitisers in medical laboratories as low as possible is likely to reduce the risk of occupational asthma. The use of low-protein, powder-free natural rubber latex or non-natural rubber latex gloves in medical laboratories will reduce airborne-natural rubber latex exposures (4). In laboratories with animals, significant reductions in airborne animal allergen levels can be achieved by 1) housing small mammals in cages with filter sheet tops or fitted filter-bonnett tops, 2) using negative pressure in cages, and 3) handling animals using a ventilated table (12). In contrast, increasing the room ventilation has little effect (12).

Appropriate occupational hygiene measures such as ventilation and use of fume hoods can reduce exposure to some chemical sensitisers and irritants. Education of workers as to the presence and appropriate handling of specific respiratory sensitisers and irritants, and understanding of the need for early medical investigation if asthma symptoms occur, may also be helpful, particularly for workers exposed to common sensitisers such as natural rubber latex and animals. Medical surveillance questionnaires (and skin prick testing for high molecular weight allergens) may also be useful in some settings in addition to reduction of airborne exposure, where there is a relatively high incidence of sensitisation and occupational asthma in order to facilitate an early diagnosis and intervention. Although there is little published data on the effectiveness of intervention measures in reducing occupational asthma from medical laboratory sensitisers, there is preliminary evidence for a benefit from general intervention measures (such as changes in glove use, education, medical surveillance) in reducing occupational asthma related to natural rubber latex (13).

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Dientamoeba fragilis. A review of a commonly detected yet poorly understood parasite of man

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Introduction and historical perspectives

"To the protozoologist – if not the physician – *D. fragilis* is now perhaps the most interesting of all the intestinal amoebae of man: for we know less about it than about any of the others, and its life history and activities are still mysterious. Ever since I first saw this curious organism in 1917, I have been intrigued by its peculiarities and have taken every opportunity of studying it further yet after more than 20 years of work and cogitation, I am still baffled..." Clifford Dobell, 1940 (1).

Although recognised by British army surgeon Lt Col Charles Wenyon in 1909, it was Margaret Jepps and Clifford Dobell who in 1917 first described and named *Dientamoeba fragilis* (2). They recognised it as the fourth amoebae after *Entamoeba coli, "Entamoeba" nana (sic)* and *Entamoeba histolytica.* Jepps and Dobell proposed the genus *Dientamoeba* because of the binuclear appearance of the trophozoites and recognised the nuclear structure alone as sufficient to distinguish it from other organisms of the genus *Entamoeba.* The species name *fragilis* was given because of its extreme frailness after leaving the body. They noted an absence of a cyst stage and reasoned by dint of the organisms lack of appearance in formed stools that it was more like a Trichomonad than an amoeba. They also did not consider it as a pathogen, although Dobell did later make a noble attempt to prove otherwise when experimenting with transmission studies on himself. He tried to orally self infect himself with a rich suspension of culture harvested *Dientamoeba* in milk. For the next 25 days he examined and cultured his stools systematically but did not acquire the infection. He then checked himself regularly for the next 10 years before he declared with modest understatement that "it is therefore certain, I think, that I failed to acquire infection with *Dientamoeba* as a result of this experiment"! (1).

Between 1917 and World War II, the few papers published on *D. fragilis* considered it a rare organism of wide distribution. Some studies however did show that it had a much higher incidence in institutionalised patients. The pathogenicity of *D. fragilis* was still not proved (a view that is still held by some workers) although it had been associated with persistent diarrhoea and abdominal distress. Original work by Burrows and Swerdlow in 1956 linked strong evidence to postulate that *Dientamoeba fragilis* was transmitted by the common human pinworm *Enterobius vermicularis* (3).

There have been approximately 80 papers published in the literature specifically on *D. fragilis* (papers listing *D. fragilis* in surveys not included); not many compared to other protozoa such as *Giardia lamblia* or, more recently, *Cryptosporidium parvum.* Latter studies have given a much better understanding of its prevalence, taxonomical status, pathogenicity and laboratory diagnosis, although many are anecdotal. Well controlled studies are lacking. Medical and many clinical parasitology texts have given scant regard to this organism despite a common occurrence world-wide and its vexatious role as an agent of diarrhoea and gastrointestinal disturbances.

Taxonomy

Dobell recognised the close structural similarities between the lumen dwelling stages of the amoeboflagellate *Histomonas meleagridis* (the causative agent of the disease 'blackhead' in poultry) and *D. fragilis.* Having reviewed the available evidence, he then came to the conclusion that *Dientamoeba* represented a stage in the life cycle of the flagellate which permanently lost its flagella (1). This close resemblance between structure and division of *Histomonas* and trichomonads was confirmed by both light and electron microscopy studies of *Histomonas* (4,5). Further, the presence of many common antigens in *Dientamoeba, Histomonas* and *Trichomonas* was demonstrated conclusively by quantitative fluorescent antibody and gel diffusion techniques (6). *Dientamoeba* was also found to be antigenically quite distinct from *Entamoeba histolytica* (7). Honigberg (8) revised the definition and taxonomic position of the species which placed it firmly amongst the flagellates, and Camp et al. (9) using electron microscopy of primarily the binucleate stage re-described the species and suggested it was placed among trichomonads in the family Dientamoebidae. Depending upon which scheme is followed, the classification is as outlined (10).

Subkingdom: Protozoa (Levine et al. 1980)	Kingdom: Protozoa (Curliss 1994)
Subphylum: Mastigophora	Phylum: Parabasala
Order: Trichomonadida	Order: Trichomonadida
Genus: Dientamoeba	Genus: Dientamoeba

D. fragilis is closely related to three species of Trichomonas affecting humans *(T. vaginalis, T. tenax, T. hominis)* and on the basis of rRNA evidence quite distinct from the other flagellates *Giardia, Enteromonas, Chilomastix* and *Retortomonas*. Classification of the protozoa is however conjectural (10). A more recent study of the sequence analysis of 16s-like rRNA established that *D. fragilis* is a trichomonad but it was not possible to explain the absence of anatomical features such as kinetosomes and flagella, axostyle, costa, pelta and nucleus surrounded by rough endoplasmic reticulum that are common to many other Parabasalia. The authors suggest that either *D. fragilis* is degenerate and has lost these features or it is truly basal in the sense that those features had not developed (11). *D. fragilis* is the only species in the genus *Dientamoeba.*

Biology and life cycle

D. fragilis is described as an amoeboflagellate, but only exists in an amoeboid trophozoite form. There is no cyst or flagellated stage. It is relatively small, being generally in the 5-12mm range, although it can vary from 3 to 22mm. Considerable size and shape variation can occur on a single smear. In fresh preparations, the organism may be actively motile, exhibiting distinct differentiation between ectoplasm and endoplasm, with hyaline pseudopodia that may be lobose or angular. Nuclei are invisible in wet preparations but food vacuoles containing bacteria may be present. There may be progressive movement. In stained preparations the trophozoite nucleus is usually seen in the typical bin-

ucleate form but may contain one and on rare occasions, three and four nuclei. On average, about 80% of the organisms are bi-nucleated and 20% mono-nucleated, but this ratio can vary considerably. The bi-nucleated forms represent an arrested telophase of mitosis, an observation originally made by Dobell in 1940 (1) and then confirmed by Bird et al. (12) and Camp et al. (9). Each nucleus characteristically consists of four to six fragmented, discrete chromatin granules but they may appear as compact bodies not unlike those seen of stained preparations of *Endolimax nana*. Peripheral nuclear chromatin is absent. The cytoplasm appearance can vary considerably. It may be vacuolated and contain ingested debris but it can also appear uniform and clean with few inclusions. It has been reported that cases of *D. fragilis* seen in appendix sections had ingested red blood cells (13) however there are no subsequent reports of this observation.

D. fragilis lives in the lumen of the caecum and upper colon, and is only found in humans. A report by Talis et al. (14) documents a 15.5% isolation rate of *D. fragilis* from gallbladder wall scrapings and duodenal juice by microscopy and culture. Talis et al. had reported gallbladder colonisation in two earlier papers, (15, 16) but no other reports are to be found in the literature that documents any site involvement other than the large intestine. All attempts to experimentally infect chicks, kittens and monkeys have failed (1, 17).

The life cycle of *D. fragilis* has been controversial since Jepps and Dobells observations in 1917. It does not have a cyst stage which is in keeping with most of the Trichomonads, but because of its apparent extreme fragility once passed into the environment, its ability to survive the passage through the gastric juices is highly improbable. (It is of interest that the name "fragilis" has had three reported interpretations. Jepps and Dobell (1) originally noted its extreme frailness after it has left the body, Knoll and Howell (17) suggested it was because the cytoplasm has a clear transparent appearance, hence its name, and Kean (18) observed a striking characteristic of the organisms exploding when distilled or tap water was added to them). Attempts to orally self transmit *D. fragilis* had failed (1) although Dobell was well aware of the similarities between *Dientamoeba* and *Histomonas* and offered the hypothesis that perhaps *D. fragilis* is transmitted from man to man in the egg of the nematode, such as *Ascaris* or *Trichuris*. The first real breakthrough as to its means of transmission was by Burrows and Swerdlow in 1956 (3) in a study of 1518 appendices. They observed that of 22 appendices harbouring *D. fragilis,* 12 were found to contain adults and/or eggs of the pinworm *E. vermicularis*. As the incidence of *Enterobius* in the appendices was 2.9% and of *Dientamoeba* 1.45% the theoretical incidence of the two occurring together should have been 0.04% however the actual incidence of the two species was 0.79%, or 20 times the expected incidence. This led them to postulate whether *Enterobius* might be a vector of *D. fragilis*. They then recovered four worms from one of the *Dientamoeba* – harbouring appendices, sectioned and stained them and examined for evidence of *Dientamoeba* within the worm. Small ameboid bodies were detected with nuclei resembling those found in *D. fragilis* from the lumen of the formalin-fixed appendices. There was also indirect evidence concurrent with this investigation when one of the authors developed light diarrhoea and abdominal discomfort, which upon examination of his stools revealed *D. fragilis* and on further examination *Enterobius* eggs. Kean (18) reviewed 100 "pure" adult infections which were free of other parasites and felt this argued against the theory that *Enterobius* plays a significant role in transmission. However Yang and Scholten's (19) analysis of 43,000 stools submitted for parasitological examination noted a combination of *D. fragilis* and *E. vermicularis* occurred 9 times more often than theoretically expected. Cerva et al. (20) examined 414 appendices and found trophozoites of *D. fragilis* in 4.8% and *E. vermicularis* in 8.75%. The co-incidence of *D. fragilis* and *E. vermicularis* was 50%. Ockert (21) experimentally infected himself with eggs of *Enterobius* from a patient who also had *D. fragilis* and subsequently developed *Dientamoeba* and another study using isoelectric point determination of *D. fragilis* in *Enterobius* eggs (22) helped confirm the interrelation between both parasites. Attempts to culture the organism from *Enterobius* eggs have not succeeded (19). *D. fragilis* has also been detected in the eggs of *Ascaris lumbricoides* (23).

The Disease

Prevalence

Jepps and Dobell in their original paper of 1917 (2) reported their findings of only seven cases (of which 3 were NZ servicemen!) out of "a very large number of persons whose stools we have examined". A literature review in 1924 found a total of 33 reported cases (24) but subsequent studies demonstrated that dientamoebiasis was common and cosmopolitan. Infection was very common in institutionalised patients with prevalence rates of 74% from a Swedish mental hospital (25), 36% from a Dutch mental hospital (26) and 42% from a Panamanian institution (27). University freshmen were found to be 4.3% infected (28) as were between 10.4% and 17.1% of navy recruits in the USA (29), and with troops returning from an Asiatic war zone 26.1% were infected (30). A large semi-communal group in Los Angeles had *Dientamoeba* observed in 52% of its members and was common in all age groups (31). Prevalence in the general population appears to be between 2%-8% (17, 20, 22, 32, 34). Figures from two New Zealand studies were similar with 2.2% (35) and 1.2% (36) respectively, although a true prevalence with the latter could not be determined because of shortcomings in detection methodology. In a Los Angeles hospital paediatric outpatient study of children in the lower to middle socio-economic group *D. fragilis* was found to be more common than *Giardia* (21% vs 17%) (37), and Preiss et al. (38) found D. fragilis in 102 of 123 paediatric patients infected by intestinal protozoa. A controlled Canadian study to determine seroprevalence of *D. fragilis* in 189 children using indirect immunofluorescence revealed that 91% of the healthy matched controls were positive at a serum dilution of 1:10 or greater (39).

Clinical presentation and significance

The role of *D. fragilis* in human disease has been controversial since its discovery. Even Dobell, the then doyen of human protozoology, was after 20 years of studying, apparently unconvinced as to whether it causes disease; "not proven" rather than "guilty" was his verdict (1). Other workers during this time were, however satisfied that infection with *Dientamoeba* does produce clinical symptoms. Weinrich et al. (28) in their survey of college students found that persons harbouring *D. fragilis* reported more gastrointestinal symptoms than those who were infected with *E. histolytica*. Sapero (29) reported that 27.3% of individuals infected with *D. fragilis* had clinical symptoms. Hood (40) reviewed 7 cases and concluded that "this amoeba undoubtedly produces gastrointestinal symptoms".

The first to obtain detailed histories from large studies were Kean and Malloch (18) and Yang and Scholten (19). The former reviewed 100 "pure" infections with *D. fragilis* from 14,000 patients over a 6 year period. The most frequent presenting symptoms were abdominal pain, diarrhoea, flatus, nausea, vomiting and fatigue. Physical signs, proctoscopic examination and radiologic studies were negative. The highest incidence according to age was in the 40-59 years group and it was slightly more common in males than females. Yang and Scholten examined 65,544 preserved stools, of which 2664 samples from 1791 patients contained *D. fragilis* alone or in combination with other parasites. They detailed the symptomatology for 255 patients in whom only *D. fragilis* was found and whom detailed symptoms had been supplied.

More than half had clinical symptoms of diarrhoea, abdominal pains or a combination of these. (Table 1)

Table 1. Frequency of gastrointestinal and other symptoms in patients in whom only D. fragilis was identified.

Symptom	% of patients				
	Yang & Scholten (19)	Literature< 1972 (19)	Spencer et al. (41) Adult	Spencer et al. (42) Children	% with symptoms + eosinophilia
Diarrhoea	58.4	42.5	68	51	75
Abdominal pain	53.7	16.2	78	60	92
Anal pruritus	11.0	2.7	12		
Abnormal stool (bloody, with mucus, loose)	9.8	22.6			
Urticaria	6.7	0			
Flatulence	5.9	19.9			
Fatigue or weakness	5.9	13.4	16	9	33
Eosinophilia	5.1	4.3			
Alternating diarrhoea and constipation	3.9	13.4	42/22	17	25
Nausea or vomiting	3.5	20.4	6	26	33
Weight loss	3.1	102		14	8
Constipation	2.4	6.5			
Belching	2.0	5.4	20		
Tenesmus	1.2	5.9		31	25
Anorexia or malaise	1.2	5.4	50		
Others	2.0	18.3		35	12
No. of patients	255	186			

A retrospective study of adult patients was made by Spencer et al. (41) who detailed the frequency of symptoms of 50 patients (Table 1) and by duration with 45. They divided the disease into acute (<7 days), sub acute (8-60 days) and chronic (>60 days) presentation. Those with acute symptoms were moderately ill with diarrhoea, abdominal pain and tenderness (especially to palpation) and nausea whereas those with chronic symptoms had abdominal pain and diarrhoea. With the latter, abdominal pain occurred 15-60 minutes after meals, was described as crampy or burning and was not relieved by antacids. Two paediatric studies (37, 38) found diarrhoea was the most common finding with acute infections and abdominal pain was most common in children with chronic symptoms. Spencer et al. (42) in a paediatric study noted that anorexia, irritability and gas were more frequent in children with *Giardia* infection whereas abdominal pain was more frequent in those with *D. fragilis*. A case of *D. fragilis* masquerading as allergic colitis was confirmed by colonoscopic biopsy of a 3 year old with chronic diarrhoea, anorexia and vomiting (43).

A gender bias with females being infected more than males has been noted (19, 20, 44, 45) and one study has observed a spring-summer seasonal increase in detection (44).

Embree and Delage from Canada in a brief paediatric review of the role of *Dientamoeba* concluded that it was a mild disease at worst and that treatment should only be considered with symptomatic children in view of the fact that chronic carriage has not been associated with any ill effects (46). In a following letter Gibbs and Church were most dismissive of *D. fragilis* as a pathogen citing the lack of good scientific evidence to support it (47). They are, however, one of the few dissenting voices. By far the majority of reports support the role of *Dientamoeba* as a pathogen (13, 17-19, 35-38, 40-45, 54, 56, 60, 66, 70, 71). Of interest in Spencer's adult study (41) was peripheral eosinophilia in 6-20%, which was significantly more common in patients with symptoms over 60 days, when compared with those with acute and sub acute illness. Low grade eosinophilia has been reported elsewhere (16, 18, 28, 38, 43), but in their study of 35 children, Spencer et al. (37) found eosinophilia present in half and was statistically more significant than in a control group. Anal pruritis has been a finding in a number of symptom descriptions, and could suggest that a concomitant *Enterobius* infection may be an explanation for *D. fragilis* associated eosinophilia, even though the worms or their eggs in most cases were not detected. Eosinophilia is not usually associated with *Enterobius* infection (48, 49, 50), nor, with the possible exception of *Isospora belli*, is it associated with intestinal protozoal infections (51). Eosinophilic colitis due to *Enterobius* has been reported (52) but is considered rare. More work is obviously required to confirm the association between *D. fragilis* and eosinophilia.

Pathology

Little has been recorded detailing the histopathology of *D. fragilis*. The first detailed study was made by Burrows et al (53) examining four cases of *Dientamoeba* infected appendixes, followed by a subsequent study by two of the same authors (13) on 11 additional cases. Of their total of 15 appendixes examined, only three had *D. fragilis* alone with *Enterobius, Trichuris, Strongyloides, Giardia, Chilomastix* and *Entamoeba coli* variously co-infecting the remaining (12). All appendixes showed fibrosis of the appendiceal wall and some showed other pathology such as lymphoid hyperplasia. Control appendixes containing combinations of the co-infecting nematodes or protozoa revealed significantly less fibrosis. They also found that in all 15 cases some of the *D. fragilis* trophozoites contained ingested red blood cells, an observation that does not appear to have been reported elsewhere. They concluded that D. fragilis probably acts as a low-grade irritant over a period of time producing a minimal injury that in turn creates a minimal inflammatory response that finally results in an increase in the fibrous connective tissue of the appendix – "analogous to a low smouldering quiet fire". In a Czech study *D. fragilis* was found in 4.8% of 414 examined appendixes but no changes to the mucous membranes were observed (20).

Dientamoeba is not considered to be an invasive organism but two reported cases of colitis due to it demonstrated a colonic wall reaction. With one case, a 27 year old woman with low grade fever, abdominal pain and multiple loose bowel movements demonstrated multiple punctate ulcers of approximately 2mm diameter at sigmoidoscopy. Biopsy revealed shallow ulceration with evidence of acute and chronic inflammation. No organisms were seen on direct saline preparations despite special stains but after polyvinyl alcohol fixation with subsequent trichrome staining many *D. fragilis* trophozoites were seen. The patient was treated with diiodohydroxyquin and recovered (54). Mild subacute eosinophilic colitis was diagnosed in a 3-year-old female patient with stool-diagnosed *D. fragilis* infection (43). She had chronic diarrhoea with mucus and leukocytes in her stools since eight weeks of age. Colonoscopy revealed an oedematous and friable mucosa extending diffusely to the splenic flexture. No ulcerations or exudates were observed. Biopsies all had a normal mucosal architecture but the lamina propria showed a moderate eosinophilic infiltrate, with the presence of hyperplastic lymphoid nodules. Microaggregates of eosinophils were observed in the crypt epithelium. The glandular architecture was normal. The patient was treated with iodoquinol, promptly became asymptomatic, and remained so after a follow-up of 18 months. The authors conclude that this case illustrates that infection with *D. fragilis* may have a clinical picture indistinguishable from allergic colitis.

Laboratory diagnosis

The trichrome or iron-haematoxylin stained film is the recommended way to diagnose *D. fragilis* (55). Although skilled microscopists examining direct saline wet preparations or iodine wet preparations from concentrates may be able to detect amoeboid bodies and perhaps

speculate as to the identification of *D. fragilis,* this organism cannot be reliably diagnosed unless a permanent stained film of an appropriately preserved faecal specimen is made. *D. fragilis* in unstained wet preparations can be confused with faecal leukocytes and other small amoebae such as E. nana. Laboratories that do not use a permanent stained film as part of their parasitology testing is probably the main reason why *D. fragilis* has been so apparently under reported. This was highlighted by Windsor and Johnson in the UK, where of an estimated 450 diagnostic laboratories they noted that most do not look for this organism (56). It is imperative that faecal specimens are also promptly preserved in a fixative such as PVA fixative or sodium acetate-acetic acid formalin fixative (SAF) as the trophozoites undergo rapid deterioration and autolysis. Most parasitology texts recommend this process as the routine procedure for all parasite examinations. A commercially available fixative and stain; EcoFix™ and EcoStain™ (Meridian Diagnostics, Inc., Cincinnati, OH, USA) also gave good results (57).

Table 2. Identification of D. fragilis

Key point microscopic identification of D. fragilis	Comments
A bi nuclear – uni nuclear ratio of 80:20 is common	Wide variation of these ratios can occur
Karyosomes fragment into 3-6 discrete dot-like particles each	May appear unfragmented and when uni-nucleated may mimic E. nana, E. hartmanni or C. mesnili trophozoites
Cytoplasm usually uniform and often has a "peppered" appearance	Ingestion of bacteria, yeasts, Sphaerita spores and other bodies as well as vacuolation can fill the cytoplasm
Usually in 9-12m size range	Large size variation can occur (5-20m)

A survey by Grendun et al (58) found that permanent staining of all stools as compared to loose or watery only, resulted in a fivefold greater detection of *D. fragilis* and recommended that all specimens, regardless of consistency, should be permanently stained. The use of a preservative is further reinforced by Chang, who observed that *D. fragilis trophozoites* die and disintegrate within one hour in an isotonic salt solution at room temperature (59). The number of organisms excreted daily has been observed to fluctuate markedly. Variations of *D. fragilis* excretion of between >15 x 106/ml to no organisms being seen was noted on daily recordings over an 80 day period in one infected individual (19). In another case history, *D. fragilis* was detected in only two of seven stool samples from a nine year old girl, which were examined on successive days (60). The distribution of organisms within a stool can also vary considerably. Greater numbers have been found in the last portion evacuated than in the first (19). The examination of three specimens collected on different days has been found to increase the yield of *D. fragilis* by 31.1% when compared with a single examination (61). An indirect immunofluorescence method to detect *D. fragilis* has been described. Antiserum, which was found to be highly specific, was raised from dixenic culture organisms (= in the presence of two defined organisms). *D. fragilis* was identified with strong fluorescence in seven of nine specimens in <1 minute each. The two specimens of doubtful readings were, on cross-reference with routine parasitology results, found to be in only very scanty numbers – less than five trophozoites on the stained smears (62). Chan et al (63) have also developed an enzyme immunoassay to detect *D. fragilis* in stools which has a sensitivity of 92% and a specificity of 87.9%. The lower level of specificity was attributed possibly to an inadequate "gold standard" of microscopy.

Neither immunofluorescence reagents or enzyme immunoassay are currently commercially available. Culture of *D. fragilis* in a "pure" state was first obtained in 1929 and is described as one of the easiest of the human intestinal amoebae to isolate and grow in vitro (1). Culture has been used by many investigators using various and mainly xenic (in the presence of other undefined organisms) methods, but the fortuitous development of the dixenic culture has been of value in antigenic research (62). Axenic culture of *D. fragilis* (= without other organisms) has been unsuccessful to date (62, 69). Culture is, however, not normally considered a practical diagnostic tool for the routine detection of intestinal protozoa (55), although it may be of value in prevalence studies (64).

Treatment

Earlier drugs for the treatment of *D. fragilis* included chiniofon, emetine, carbarsone and diphetarsone, all of which were reported to clear the organism (17, 18, 40, 65), but which have potentially serious side effects. Over recent years five other drugs have been regularly used in the treatment of D. fragilis. These are iodoquinol, metronidazole, paromomycin, diloxanide furoate and tetracycline. Iodoquinol (diidohydroxyquin – Yodoxin [Glenwood], others) a halogenated oxyquinoline, is regarded by many as the drug of choice (31, 37, 41, 42, 67, 70). Being poorly absorbed it acts as a luminicide and is tolerated well, provided dosage and duration are not exceeded. Metronidazole (Flagyl [Searle], others) is a nitroimidazole and is rapidly absorbed after oral administration. It probably does not reach therapeutic concentrations in the large intestine and is not recommended as a cyst eradicator (68). Preiss et al. (38) found metronidazole in a paediatric study was not effective in 30% of patients, and Oxner et al. (35) noted that treatment with metronidazole had an unpredictable outcome. Nausea, headache, metallic taste and anorexia are frequent side effects. Paromomycin (Humatin [Parke-Davis]) is a poorly absorbed aminoglycoside with most of an oral dose excreted unchanged in the faeces. It is effective against *E. histolytica/dispar* cysts (68). Diloxinide furoate (Furamide [Boots]) is a well tolerated luminicide and is also used to eradicate *E. histolytica/dispar* cysts (68). Tetracycline is regarded as a "safe" and efficacious drug (66), although it does have the disadvantage of not being able to be used in treating children or pregnant women (67).

Table 3. Recommended drug regimens for D. fragilis (67)

Drug	Adult Dose	Paediatric Dose
Iodoquinol	650mg tid x 20 days	40mg/kg.day (max 2g) in 3 doses x 20 days
Paromomycin	25-30mg/k/day in 3 doses x 7 days	25-30mg/k/day in 3 doses x 7 days
Tetracycline	50mg qid x 10 days	>8 yrs of age: 40mg/kg/day (max 2g) in 4 doses x 10 days

A problem affecting the treatment recommendations for *D. fragilis* infections is the lack of randomised double blind clinical trials. All reports in the literature are anecdotal. Chan et al. (69), using a single isolate, performed experimental minimal amoebicidal concentrations (MAC) to iodoquinol (MAC = 128mg/ml), paromomycin (16mg/ml), tetracycline (32mg/ml [questionably]) and metronidazole (32mg/ml), and this appears to be the only *in-vitro* testing of susceptibility that has been made to these drugs.

Conclusions

Dientamoeba fragilis has proved to be an enigmatic organism taxonomically, epidemiologically and pathologically. Its position amongst the Trichomonadidae has been confirmed by electron microscopy, antigen studies, and more recently by molecular methods. Described as an unflagellated flagellate, the genus name *Dientamoeba* is literally a misnomer because, although it is amoeboid in appearance, that is where the similarity ends.

Its life cycle is unique amongst the protozoa infecting humans,

although there is a proved animal example of such a cycle with a flagellate of poultry, *Histomonas meleagridis* being paratenically transmitted by the nematode *Heterakis gallinae*. Evidence that *D. fragilis* is transmitted by the common pinworm *Enterobius vermicularis,* and possibly some other nematodes, is well documented. The higher than expected coincidence of *D. fragilis* and *E. vermicularis* infections, microscopic evidence of the protozoan in pinworm eggs, and the similarity of isoelectric point comparison of *D. fragilis* in culture and those in the eggs is all compelling evidence. This is even made more so when a documented self-inoculation experiment of pinworm eggs, as well as anecdotal reports of *Dientamoeba* researchers who themselves have contracted the infection, have monitored its progress to confirm the nematode link. What is not clear, however, is whether a direct faecal-oral route or similar can also occur under certain circumstances. The lack of a cyst stage, some simple observations (the results of which give support to the species name "fragilis"), and Dobell's experiment of self inoculation of the trophozoites indicates that it cannot survive the stomach-small intestine journey to the colon. This would also be in keeping with the inability of the trophozoite stages of other protozoa to initiate infection, the possible exception being *Trichomonas (Pentatrichomonas) hominis.* This intestinal trichomonad also does not have a cyst stage, yet it is assumed that its trophozoites are transmitted by faecal – oral means, and survive the gastric barrier.

Enterobius is a very common if not a nearly universal infection of children at some stage in their early lives, which supports the nematode lifecycle involvement. Many adults likewise acquire *Dientamoeba,* but do not have an (evident) pinworm infection, and this also raises the possibility of another means of transmission. It could, however, be with these cases that *D. fragilis* may have been in a quiescent state in adult patients for some time before symptoms developed, and Enterobius infection may have gone unnoticed or had spontaneously resolved, although there is no evidence to support this postulation.

Is *D. fragilis* a pathogen? Despite the occasional paper casting doubts, the majority of evidence and general consensus is that it is a cause of diarrhoea and/or abdominal pain with both an acute and chronic presentation. Chronic carriage without any symptoms may occur. Almost all evidence suggests large intestine colonisation only. The few papers (from the same set of authors) documenting gallbladder involvement have never been substantiated. Although *D. fragilis* does not invade the colonic wall, histopathology has revealed superficial tissue involvement in some cases.

Treatment of dientamoebiasis remains a very neglected aspect of the disease. There is very little experimental data on drug treatment, and blinded trials, including those using combination therapy, need to be conducted. A standardised *in vitro* drug testing method using strains grown axenically needs to be developed to assess drug efficacy. Two of the four drugs of choice, iodoquinol and paromomycin are not registered for general use in NZ and only issued to individually named patients. They are only held by some hospitals. Diloxinide furoate is no longer available in NZ.

The recognition and identification of *D. fragilis* should pose no problems for diligent medical laboratory scientists provided immediate faecal preservation is made (preferably at the point of collection), and a permanent stained film from it is examined. Multiple specimens collected on different days may be required. EIA testing, when commercially available, and particularly if it can be combined with the currently available *Giardia* and *Cryptosporidium* test kits, may offer a good alternative to microscopy in certain settings. *D. fragilis* is an organism with a chequered history that has taken a long time to be recognised by the medical fraternity as a gastrointestinal pathogen. It should now be considered on equal terms with the other more established protozoa that can cause disease. Controlled research into its epidemiology, pathology and treatment is well overdue.

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An evaluation and comparison between blood film microscopy services in government hospital laboratories in the Mid-Western Region of Nepal and the Waikato Region in New Zealand

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Abstract

Introduction: An evaluation of blood film microscopy services was carried out in the Mid-Western region of Nepal and the Waikato region in New Zealand to define strengths and weaknesses, and thereby further enhance the quality of service provided.

Methods: Questionnaires and blood films compared qualifications and experience of staff, training resources, technique in preparation, examination, reporting and referral of blood films, and participation in and assessment of the external quality assurance program utilised.

Results: Evaluation of the two regions with international recommendations, revealed that while many technical aspects are similar, training and resources are limited in the Mid-Western region. In the Waikato staffing is higher, specialised staff are easily accessible and abnormal films are regularly examined. Examination of blood films between the regions revealed a lack of accuracy in the Mid-Western region, while external quality assurance (EQA) programs with an emphasis on education are of great benefit to laboratories in maintaining competence.

Conclusions: The ability to utilise technology and computers is very limited, and increasing staff numbers is difficult to achieve in Nepal in the immediate future. However, improving resources and training of staff can be achieved through regular refresher training, morphology workshops, distribution of abnormal films with educational supplements, and supply of blood atlases and sets of teaching slides. In New Zealand it is important for small satellite laboratories to maintain quality through regular participation in EQA programs and morphology workshops, and blood atlases and sets of teaching slides should be utilised frequently.

Key words: microscopy, differential leukocyte count, quality control

Introduction

Blood film microscopy (blood smears, leukocyte differential count, examination of a blood film specifically for normality or abnormality of white blood cells, red blood cells and platelets, but not specifically for malaria or microfilaria), is a basic fundamental laboratory procedure that can easily be utilised in any routine medical laboratory throughout the world. It serves as a diagnostic tool for doctors and other health professionals, as a tool for monitoring disease state, and as a quality control tool in verifying the results generated by automated analysers.

This study reviews international standards of blood film microscopy and compares those to routine laboratory life in a region of a developed country (New Zealand) and a developing country (Nepal).

It was not until after 1950 that modern development came to Nepal, one of the poorest nations in the world with a per capita GDP of US$200 and population of 21,000,000 people (1). The resource poor nature and geography of the country limit development of health services. National objectives for laboratory services target improvement of overall quality control, ensuring sufficient manpower is trained for laboratory work, and ensuring there are sufficient laboratory supplies (2). For three years International Nepal Fellowship, an international non-government organization (INGO) has been assisting the Nepalese government in its mandate to strengthen laboratory services. Initial surveys of government laboratories revealed a lack of resources, training and quality of laboratory results.

New Zealand boasts a population of 3,600,000 people (3), in which laboratory services play a key role in health care. Over the last 10 - 15 years health reforms have attempted to increase efficiency and improve standards, becoming customer orientated in nature.

In New Zealand all medical testing laboratories are assessed for accreditation against a combination of the international standards ISO 9002 (quality management systems) and ISO Guide 25 (technical competency), but there are no formally documented standards for blood film microscopy evaluation. This study assesses blood films similar to guidelines of International Accreditation New Zealand (IANZ) (personal correspondence, IANZ by considering the following items:

- Qualifications, experience and competency of staff performing microscopy
- Standard of textbooks, morphological atlas and other reference material
- Criteria for preparation and examination of blood film
- Specimen collection and blood film preparation
- Staining, examination and reporting of blood films
- Referral of a film onto a pathologist or laboratory colleague
- Participation in inter-laboratory comparison programme

In addition, in 1997 and 1998, in both regions being examined, an external quality assessment (EQA) program was started for blood films, which is included in the evaluation. This examination will highlight strengths and weaknesses and thus provide information for further strengthening and development of laboratories.

History

Blood film examination has evolved significantly over the centuries from the discovery of magnifable cells to a rapidly evolving technological age. According to Groner and Simson (4), the history of blood cell analysis can be divided into roughly four phases:

1. Discovery (1642-1881). Leeuwenhook first noted the cells of the

blood in 1642. Notable events included a) the discovery of platelets by Donne, in 1842, and b) the differentiation between lymphocytes and granulocytes by Gulliver in 1846. At the end of the 19th century Paul Ehrlich applied analine dyes.

2. Application (1881-1950). Microscopic observations and the quantification of the cellular elements of the blood were related to clinical phenomena. Important discoveries during this phase include a) relating the morphology of neutrophils to infection and inflammation and b) a detailed classification of leukemia's on the basis of morphology.

3. Consolidation (1951-1965). It was well recognized that manual blood film microscopy was tedious, expensive, time consuming, and results were relatively inaccurate therefore main developments of this phase focused on automation technology and cost reduction.

4. Rediscovery (1965 to present time). This current phase has focused on observing with more specific chemistries the molecular structure of the cells, bridging what is observed microscopically at the cellular level in blood to what is found clinically. Developments in this phase have enabled alternatives for leukocyte classification.

During the 1970s extensive efforts were made to automate the leukocyte differential count (5). The last fifteen years have seen a rapid evolution of technology (6), to the extent that latest systems perform not only the complete blood count, and differential leukocyte count, but also makes and stains the blood films, etc (e.g. Coulters GENS). The flagging systems have decreased the workload and cost in the microscopic section significantly, however manual blood film microscopy remains a core part of the haematology laboratory.

Blood film processing

The need for a well-made and well-stained blood film is imperative in the examination and subsequent reporting of results.

A. Criteria for preparation and examination of blood film

Flagging of abnormal parameters by automated cell counters provides selective blood film examination (7). Studies conducted by the General Haematology Task Force in the United Kingdom (8), assessed the role and need for manual blood films to be examined with automated blood counting systems. One study examining flagging criteria - clinical flag, instrumental flag, delta check flag, confirmed that the higher the percentage of the films examined, the less likely it is to miss significant pathology. The task force concluded that an intelligent balance between the minimum risk and the maximum laboratory efficiency should be sought, taking into consideration, workload, staffing, and local expectations.

B. Specimen collection and blood film preparation

The H20-A document approved by the National Committee for Clinical Laboratory Standards (NCCLS) in 1992 (9-11), states that whole venous blood collected in tripotassium ethylenediamine tetra-acetate (K3EDTA) is the required specimen for film preparation. Alternatively, blood collected by skin puncture maybe used.

Three different techniques for blood film preparation have been described - the wedge-pull technique, the coverslip method, and the spinner method. All three methods are well documented, however in today's laboratory the wedge smear is the most popular and recommended by the NCCLS (12-17). The blood film should be labelled appropriately before staining

C. Staining

The NCCLS H20-A document advises the film should be stained within one hour of preparation, or fixed within one hour with "water-free" methanol. This reference method for blood film staining recommends the use of a Romanowsky stain. A Romanowsky stain consists of a mixture of azure B and usually eosin B or Y dyes, at pH 6.4-7.0. The reference method advocated by the International Committee for Standardisation in Haematology (ICSH) prefers the use of pure azure B and eosin Y, giving inter-batch consistency and standardisation. Successful staining enables accuracy in identifying mature and immature leukocytes and abnormal cells (18-19).

Several Romanowsky stain combinations are documented for use in blood film staining, including Wright, Giemsa, Wright-Giemsa, May-Grunwald-Giemsa, Jenner-Giemsa,Leishman and Field's (the latter for rapid staining) (20-26). Films can be stained either by manual or automated methods.

D. Examination

Blood film examination has been extensively reviewed by Barbara Bain and Dacie and Lewis (27). Blood films should be examined systematically.

First, patient identification must be checked and the blood film matched with the appropriate blood count report. At this stage the patient's sex, age, ethnic origin and clinical details should be noted.

Next, the film should be examined macroscopically and microscopically observing adequacy of preparation and staining, and cell morphology. After this initial assessment, if the blood film is deemed unsatisfactory, a fresh film should be prepared and stained.

The film appearances should then be compared with the full blood count and judged whether results are consistent. Discrepancies due to technical errors or abnormality of the specimen must be addressed.

For daily routine work a 100-cell leukocyte differential count is then performed. Three counting techniques have been described in an attempt to compensate for maldistribution of cells. These methods include tracking along the length of the film, the modified battlement method and the battlement method on a wedge made film (the latter being the NCCLS recommendation).

The area of the film is found where erythrocytes lie in a mono-layer. Each identified leukocyte cell must be placed into the appropriate category (e.g segmented neutrophil, blast cell, etc). Two methods of counting nucleated erythroid cells are documented – counting nucleated erythroid cells with leukocytes or per 100 leukocytes counted. As much has been documented on the decreased accuracy and precision of a manual differential count than an automated count, a manual count should therefore only be performed if indicated (28-31).

Subsequently morphological appearances of leukocytes, erythrocytes and platelets are assessed and commented on. Assessment of leukocytes should include examination for a left shift, variant lymphocytes, plasma cells, abnormal myeloid changes (e.g. hyersegmentation, pelger-huet anomaly), toxic changes (e.g. toxic granulation, Dohle bodies), pyknotic cells and smear cells. Erythrocyte examination assesses morphological variation in size (e.g microcytes, macrocytes), colour (e.g. hypochromasia), shape (e.g. acanthocytes, elliptocytes), inclusions (e.g. howell jolly bodies, pappenheimer bodies) and distribution (e.g. agglutination, rouleaux). Assessment of platelet morphology includes platelet numbers, size, granules, platelet-neutrophil satellism, and platelet clumping. The thorough laboratory worker should also examine the blood film for parasites (e.g. malaria, microfilarie).

Clinical details, sex, age, ethnic origin, blood results, differential count and all morphological features should then be correlated to provide a useful report for the clinician.

E. Reporting

Reporting of leukocyte differential counts continues to be controversial. Traditionally, counts were reported as a percentage, as the data were directly available from the blood film. From a mathematical perspective, it would appear there are advantages to both absolute and

percentage reporting. Britain tends towards reporting absolutes while the United States continue to report percentages, reflected in the NCCLS document. Koepke et al advocate the reporting of absolute counts, suggesting it offers a more sensitive indicator of the patient's clinical condition.

In New Zealand the Auckland Haematology Special Interest Group (HSIG) published a booklet establishing standard nomenclature and expression of results (32). Morphological features maybe graded 1+ through 4+ or as slight, moderate, or marked, etc. It is recommended, reporting of abnormal morphology be accompanied with an interpretative comment where possible.

Generation of reports using interpretative reporting may include marking abnormal results, printing reference values, suggesting diagnostic possibilities, and making recommendations for which additional studies may establish the diagnosis.

Computer technology has revolutionised reporting and data transmission of results, whereby cumulative results are quickly available, minimising cost and turnaround time of results, improving consistency of reporting and therefore improving patient care.

F. Referral

Access to more specialised centres are important for laboratory workers, for the referral of abnormal blood films for confirmation of findings, diagnosis or follow-up of haematological abnormalities. Smaller laboratories need access to regional and national laboratories. Blood films needing specific expertise (e.g. Classification of malaria or microfilaria), should be referred to specialised laboratories.

External quality control and continuing education

Quality control of manual blood films does not so much assess technical performance as much as the competence of the laboratory worker performing the test. External quality assessment (EQA) of blood films, is an extension of internal quality control (IQC). It provides the facility for training staff in recognition and interpretation of morphological features and it is a means for assessing the professional skill of staff. The WHO defines EQA as "a system of objectively checking laboratory results by means of an external agency...to establish between-laboratory comparability" (33).

Blood film assessment is an integral part of many EQA programmes throughout the world (e.g. Survey program of the College of American Pathologists (CAP), the United Kingdom national external quality assessment scheme (UK NEQAS), WHO program in collaboration with UK NEQAS, and the Royal College of Pathologist's of Australasia scheme (RCPA). Details of some of these programs are well documented (34-38).

Some important aspects of establishing and running an EQA program include:

1. Establishing confidence between participants and organisers is essential and can be gained by allowing voluntary participation until confidence has been established, considering the organiser as an advisor, never an investigator and making physical inspections completely independent of EQA.
2. Material distributed should resemble specimens from patients and include both normal films (for differential cell counting) and abnormal films (including rare conditions).
3. Stained and covered slides or 35mm transparencies are better as it has been discovered that delays beyond 6-8 hours in staining the film may result in unsatisfactory staining.
4. Specimens should be distributed at least four times a year but not more than every 2-3 weeks.
5. The films can be accompanied by the blood count with brief clinical notes and basic blood results, although controversy surrounds how much 'leading' information should be given.
6. Individual reports should be made confidential.
7. It is unrealistic to identify poor performance by analysis of differential counts due to the wide deviation of cell distribution, although when cells are mis-classified (e.g. lymphocytes are counted instead of blast cells) a differential count can be valuable. Some schemes (e.g. UK NEQAS), do not include poor performance in the assessment, as they are regarded as educational.
8. The time elapse before return of the analysis of the participants' performance should be as short as possible.
9. EQA should be educational with the main aim to help participants' improve their performance. A wide selection of blood films will help to stimulate and challenge the participants, provide educational material and to give participants an opportunity to build up slide libraries. Continuing education can be provided through the program (e.g. CAP program) or as morphology workshops (e.g. ASCP, New Zealand haematology SIG) (Personal Correspondence. American Society of Clinical Pathologists). For developing countries, UK NEQAS WHO collaborating centres as well as operating international EQA, provide education, training and consultancy.

Materials and methods

Study subjects

A representative group in Nepal and New Zealand were chosen as study subjects (government hospital laboratories in the Mid-Western region and Waikato region respectively). Staffed government hospital laboratory numbers are similar and both regions have established EQA programs for blood films in the last three years.

The Mid-Western region is the poorest region in Nepal. Of the ten hospitals in the region, only six have laboratory staff. Throughout the region, there are 60% laboratory staff vacancies in government hospitals. No cell counters are available and reporting is written. Official staff hours are 10am – 3pm. Private practice engulfs the remaining hours. The population of the Mid-Western region is 2,909,753 persons.

The Waikato region in New Zealand accommodates one referral hospital and four-government satellite hospitals. Each of these laboratories is automated, with the satellite laboratories accommodating the same model of cell counter. This assists with comparability and staffing across laboratories and cost-effectiveness. Computer link with the referral hospital will also be very advantageous when completed. In 1996 the population of the Waikato region was 350, 124.

Methods

Between March 1999 and January 2000, six government hospitals in the Mid- Western region were visited. Four government hospitals in the Waikato region were visited between May and June 1999 (questionnaires and blood films were posted to the fifth hospital). Two questionnaires assess routine blood film microscopy and the blood film EQA program. Four blood films and a result sheet were distributed for a comparative assessment.

1. Laboratory questionnaire (Appendix 1)

Where it was possible, the questionnaire was filled out with the laboratory staff. In Nepal project laboratory colleagues assisted in filling out the questionnaire and with translation. The questionnaire was translated into Nepali and available for the government laboratory staff to refer to if questions were difficult to understand.

To assist with questions regarding training of medical laboratory scientists in New Zealand, haematology lecturers at Massey University and Auckland Institute of Technology were interviewed and a letter sent to Otago University.

2. Quality control program evaluation

Evaluation of the regional Quality Control programs operating in the Mid-Western region and the Waikato region comprised of two components – a questionnaire designed for program organisers (Appendix 2) and a questionnaire designed for program participants (Appendix 3).

3. Comparative quality control blood films

Twenty wedge blood films were made from four different EDTA samples and labelled as QC 1, 2, 3 and 4. After drying they were fixed immediately and all QC 2 and 3 films and 3 slides each from QC 1 and 4 were stained using May-Grunwald Giemsa in an automated staining machine. One slide of each QC sample was given to the laboratories in the Waikato and Mid-Western region. Participants were instructed how to process the slides (Appendix 4). The blood smears were designed to reflect the quality control programs between the two regions. (i.e. two smears stained with information, two smears unstained without information).

Leukocyte differential count

Competent laboratory scientists from one referral hospital and the author performed a total of ten 100-cell leukocyte differential counts on each of the four lots of quality control films, using the automated stained slides. The differential counts were put into a spreadsheet in Microsoft Excel. The averages were calculated on the ten reference counts for each lot of quality control, and these became the target values. The target values were plotted on a bar graph, against the participating laboratories' results, for each differential. Statistical analysis would have proved inaccurate due to lack of volume of data, therefore was not performed.

Film and interpretative comments

Both film and interpretative comments were collated into a table in Microsoft Word. These were then viewed for any blatant inconsistencies.

For each blood film a target result was set as follows:

QC1 - left shift detected

QC2 - counting of blast cells with diagnosis of leukaemia or referral

QC3 - increased number of monocytes, target cells detected. (Beneficial if, dimorphic or hypochromic cells detected and diagnosis of liver dysfunction).

QC4 - detection of toxic changes in neutrophils, microcytosis of red cells, fragmented cells and thrombocytopenia.

In all cases, if a result was reported as needing referral, this was acceptable.

A final table comparing each laboratory against the above target criteria was compiled for direct comparison between regions.

Results

Laboratory questionnaires

In the Waikato region, four out of the five government hospital's participated in the survey, and in the Mid-Western region six out of six participated. Tables 1 and 2 show a higher proportion of doctors and hospital's per population in the Waikato and a high proportion of highly qualified medical laboratory scientists. In the Mid-Western region staff shortages are common and the level of training of staff examining blood films is lower.

1. Qualifications, experience and competency of staff

Blood film training included six weeks of basic training followed by supervision, for hospital trained medical laboratory scientists in New Zealand, being competent to perform blood film microscopy by the end of the training. The four-year training courses now offered at Otago and Massey Universities, and at Auckland University of Technology, for Medical Laboratory Science, students begin study of blood films in either their first or third year with a significant portion of practical sessions committed to blood film examination. Haematology graduates should be able to perform examination on a blood film. In Nepal a laboratory technician receives a two-year general training while an assistant may have received a one year general training, or have been given a three month training in malaria examination. Most staff in the Mid-Western region did not feel the initial training for blood film examination was adequate and wanted refresher training.

Competency of Waikato regional staff is assessed by a senior staff member, through an evaluation / coaching process, discussion and by participation in surveys. Competency documents record each staff member's ability. It was felt amongst some staff in the Mid-Western region that competency was assessed from the national quality assessment program.

In the Waikato, supervision is given internally by senior staff, and externally by the IANZ accreditation and surveillance visits, or a visit from a haematologist. External supervision visits are linked with the national quality assurance program and Tuberculosis program in the Mid-Western region, while internal supervision visits are from the hospital superintendent.

Table 1. Details of surveyed hospitals.

Lab	Region	No. of beds	No. of Dr's	Consult. Haematologist	Pop. Served	Blood films per month
Lab A	Waikato	700	307	Yes	300 000+	2200
Lab B	Waikato	23	2	Referral	5000	150
Lab C	Waikato	28	4	Referral	10 000	180
Lab D	Waikato	55	9	Referral	60 000	820
Lab E	Mid-West	35	1	No	279 355	50
Lab F	Mid-West	15	4	No	436 310	400
Lab G	Mid-West	15	0	No	368 456	160
Lab H	Mid-West	15	1	No	203 830	240
Lab I	Mid-West	15	1	No	219 456	35
Lab J	Mid-West	150	20	No	361 702+	720

Notes: NB: The population served in the Mid West is the population of the District only. As many districts do not have doctors, patients are referred to these districts with doctors and hospitals. Districts without hospitals amount to an added population of 952 375.

Table 2. Staffing of laboratories

Laboratory	No of staff in the dept	Staff vacancies	Adequacy of staffing	Training of staff doing films
Lab A	13.2 FTE	0	Adequate	Technologist
Lab B	1	1	Adequate	2 Technologist 1 Assistant
Lab C	1.5 FTE	0	Adequate - overstaffed	2 Technologist 1 Assistant 1 QTA
Lab D	1.5 FTE	0	Understaffed	2 Technologist
Lab E	3	1	Understaffed	1 Technician 2 Assistant
Lab F	3	1	Understaffed	1 Technician 2 Assistant
Lab G	2	2	Understaffed	1 Technician 1 Assistant
Lab H	3	1	Adequate	1 Technician 2 Assistant
Lab I	1	3	Understaffed	1 Assistant
Lab J	5	1	Adequate	1 Technician

Notes: *FTE – full time equivalent is the total number of staff working including part-time and casual staff. This measure is used in New Zealand but not in Nepal.

QTA – qualified technical assistant

2. Continuing education
Due to the high number of qualified medical laboratory scientists and haematologists at the referral laboratory in the Waikato region, refresher training is an ongoing internal activity. Staff also have opportunities to attend a morphology workshop, as do other staff from the smaller satellite laboratories. Most staff in the Mid-Western region have received no refresher training, and if they have, it was a general laboratory training and not specifically for blood films.

Only one laboratory in the Mid-Western region reported having access to resources to assist them in blood film examination (e.g. blood atlases, teaching slides, etc). In contrast, in the Waikato all laboratories have atlases / reference books, teaching slides that are continually being added to through external quality assessment schemes, and internet access.

3. Criteria for preparation and examination of a blood film
In the Waikato a blood film could be examined manually:
a) as requested by a doctor
b) based on instrument results and flags
c) based on clinical details
d) if a significant change in previous results has occurred
e) due to findings on the previous blood film

In some laboratories however blood films are examined on all specimens. Contrasted to this automated approach, in the Mid-Western region a blood film is ever only examined manually if a doctor orders the test.

4. Specimen collection and blood film preparation
All laboratories in both countries use wedge films, however in the Waikato blood films are usually made from EDTA blood, while in the Mid-Western region it is common practice for staff to make blood films directly from a skin puncture.

5. Staining
The Rowmanowsky stains used in the Waikato are either May-Grunwald Giemsa or Wright-Giemsa while in the Mid-Western region all use Wright stain (one also used Leishman's stain). All laboratories have an automated staining machine in the Waikato and all stain blood films manually in the Mid-Western region.

6. Examination
All laboratories agreed that in evaluating the adequacy of the film preparation and staining, the film should be assessed both macroscopically and microscopically. If it was found inadequate in any way the film should be remade.

Computerisation and automation enables staff in the Waikato to access all laboratory results, including previous results to assist in film examination. In the Mid-Western region however staff have access to only those results performed on the same day.

A significant difference between both regions, is that in the Mid-Western region only a 100-cell differential count is performed, whereas in the Waikato a manual 100-cell differential count is usually only performed if review of the film indicates an incorrect or anomalous automated differential. Further, in the Waikato, white cells, red cells and platelet morphology is assessed.

7. Reporting
In the Mid-Western region differential percentages are always reported while in the Waikato differential absolutes are always reported and sometimes percentages in addition. Cell comments and interpretative comments are much more a feature on computer generated customer orientated reports in the Waikato.

8. Referral
In the Waikato all laboratories had a colleague to confirm abnormal findings if needed (e.g. medical laboratory scientists, haematologist) whereas in the Mid-Western region three of the six laboratories had no able colleague. Satellite laboratories in the Waikato referred abnormal films and sometimes parasites onto the referral hospital in the region, while the referral hospital review difficult films by group assessment of medical laboratory scientists and haematologists before a definitive comment is reported. Malarial smears may be referred onto a reference laboratory.

Mid-Western region staff refer results onto the hospital doctor and occasionally a pathologist in Kathmandu.

9. External quality assurance participation
All laboratories in the Mid-Western region are expected to participate in the national external quality assurance program organised from the National Public Health Laboratory in Kathmandu. Waikato laboratories participate in the program organised by the referral hospital in the region, the RCPA survey program or they receive the check samples from the ACSP, or a combination of all three. Internal quality control was assessed by ongoing monitoring from senior staff and validation of film and interpretative comments with complete blood count results at sign-out of reports.

Quality assurance program evaluation
In the Waikato the aim is to enable rural hospital laboratories to compare their results with each other and a central reference laboratory, to expose staff to blood film appearances rarely seen, to identify poor performance and as an educational tool. Waikato regional participants find the program beneficial in maintaining professional confidence and competence, and the ongoing education provides stimulation and challenge.

In the Mid-Western region the program is a means to assess performance and identify poor performance for follow-up refresher training, and thereby improve laboratory standards. Participants find the program beneficial to assess the accuracy of their results, and for comparison against other laboratories. It was noted however that due to the age of the blood films, staining was difficult.

A comparison can be seen between the two programs in Table 3. Data is insufficient for statistical analysis in both programs, therefore neither program analyse data statistically. However figures 1-2 do show that on a normal blood film participants' results in the Waikato are comparable, while in the Mid-Western region there is a wide spread of results (Fig. 3-4).

Comparative quality control blood films
In the Waikato region, all four participating laboratories examined and reported results. Results were reported by medical laboratory scientists. In the Mid-Western region, four of the six laboratories participated. Laboratory assistants only reported a differential count, while laboratory technicians also attempted reporting a film comment, and diagnosis. Interestingly, all these staff counted only normal cells and reported immature white cells if seen.

Leukocyte differential count
Figures 5 – 8 show the four blood film results in graph form, from all the participating laboratories. Figure 5 shows that Waikato staff detected a left-shift, but most staff in the Mid Western region did not. Figure 6 shows all laboratories in the Waikato region counted blast cells, compared to none in the Mid-Western region, although one laboratory reported seeing immature white blood cells. Figure 7 shows Waikato laboratories counted an increase in monocytes, Mid-Western

Table 3. Results of the EQA programs

	Waikato Region	Mid-Western region
Voluntary Participation	Yes	No
Organisers are advisors / inspectors	Advisors	Advisors and inspectors
Physical inspections	Not related to the quality control program	Supervision is an integral aspect of the program
Normal and abnormal films	A wide selection of normal and rarely seen films is distributed	Normal films only
Staining	Pre-stained May-Grunwald Giemsa films and cover slipped	Unstained fixed films are distributed
Frequency	Every week	Two films every year
Clinical notes	Clinical details, age, sex, Hb, MCV, WBC, Plts	No details included
Differential count	Sometimes required	Always
Cell morphology Comment	Sometimes required	No
Interpretative Comment	Usually required	No
Confidentiality of reports	No	Yes
Analysis	All participating lab results are collated together, photocopied and posted. No statistical analysis.	All participating lab results are collated together, photocopied and posted. No statistical analysis.
Electronic transfer of results	Possible	Not possible
Time elapse of reporting	Two weeks	Two months
Performance assessment	1.Poor performance is noted (e.g. lymphocytes counted instead of blast cells). 2.Consistent poor performance may result in a phone call to the laboratory, with the offer of refresher training. 3.Lack of participation is noted.	1.Poor performance is noted by organisers (e.g. wide spread of results from target results). 2.Follow-up includes review of blood films on next visit to the laboratory, and systematic refresher training of all staff.
Educational	A weekly educational supplement is included. Enables labs to build-up a teaching slide set. Refresher training is offered, but no morphology workshop yet	Half day of training – practical and theory, in general lab refresher courses.
Limitations	1.Finding bloods with new appearances and not repeating similar blood film findings too frequently. 2.Organisation is time eansuming	1.Motivation of staff 2.Geography 3.Insufficient staff to properly implement the program.

region laboratories did not detect this. Results from blood film 4 (figure 8) indicate a high percentage of neutrophils, yet in the Mid-Western region most laboratories reported relatively normal results. In addition, it can be seen from all these graphs, that there is a wide variation between results from the Mid-Western region.

It was noted that staining of the unstained blood films (1 and 4) in the Mid-Western region was inadequate for proper examination. There was a delay of seven months, between film preparation and distribution.

Table 4 highlights the significant differences between the two regions. Laboratories in the Waikato region achieved reporting most of the target results required, whereas in the Mid-Western region, not many of the target requirements were reported. This aberrance can be attributed to the lack of film comment and diagnosis reporting.

Discussion

It is clearly seen that blood film morphology services in the Mid-Western region are currently in the 'pre-1950's' era, while services in the Waikato region are 'state of the art'. The implications of resources or lack of them, is seen through the quality of service available.

In both regions, many aspects are comparable. While specimen collection varies in each region, both techniques correlate with international recommendations. Wedge smears are made and, Rowmanowsky stains used, however staff, particularly in the Mid-Western region using manual methods, need to be aware of maintaining good technique. Examination of the actual film is similar, but more information is available for staff in the Waikato region. Availability of patient data, clinical details and laboratory results, including a complete blood count profile, significantly aid in examination and fuller reporting, ultimately assisting in patient care.

This in turn is related to resource availability. Laboratory staff, doctors, health workers and patients in the Waikato region benefit from state of the art computer systems and analysers. There is greater

Table 4. Comparative quality control study

	A	B	C	D	E	F	G	H	I	F
QC1 – left shift	Y	Y	Y	Y	N	Y		N	N	
QC2 – blast cells	Y	Y	Y	Y	N	?		N	N	
- leukaemia / referral	Y	Y	Y	Y	N	Y		N	N	
QC3	Y	Y	Y	Y	N	N		N	N	
- monocytes										
- target cells	Y	Y	Y	Y	N	N		N	N	
- (dimorphic/ hypochromic)	N	Y	Y	Y	N	Y		N	N	
- (liver dysfunction)	Y	N	N	Y	N	N		N	N	
QC4 – toxic changes	Y	Y	Y	Y	N	N		N	N	
- microcytes	Y	Y	Y	Y	N	N		N	N	
- fragmented cells	Y	Y	Y	Y	N	N		N	N	
- low platelets	Y	Y	N	Y	N	N		N	N	

Notes: Y = yes N = no

flexibility for extra tests to be added if indicated to assist patient diagnosis, while government subsidy of laboratory tests are a great advantage.

This contrasts sharply to the Mid-Western region, where staff perform only the tests requested, and the patient pays for each test. While blood film examination is under-utilised in the Mid-Western region, and the quality of service is lower than in the Waikato region, it is important to acknowledge that in improving the quality, it should be appropriate for the situation. For many diseases, there is no, or limited treatment, and therefore the importance to recognise different disease states in a blood film for diagnosis is not as great. In addition, as described earlier, automation of the leukocyte differential count was invented not only to improve quality but also as a cost saving tool. In Nepal, where staff salaries are cheaper, it is more cost-effective to perform a manual differential leukocyte count than install automation. This however does impede quality.

Reporting of results from the comparative study and the quality assurance programs, show clearly the lack of quality in the Mid-Western region. It can also be seen, however, that the situations in which the staff work, are less than optimal compared to the Waikato laboratories. Reasons for this lack of performance include limited training in blood film morphology, low staffing not allowing for colleague stimulation, coaching or referral, staff working in isolated situations, limited continuous education and lack of specialised staff. To improve the quality of blood film morphology there is clearly a need for further training of staff, covering adequate staining, examination of techniques and assessment of blood film components. Providing refresher training, distribution of blood atlases and teaching slides, and regular examination of abnormal films will also improve staff competency, and therefore quality.

In New Zealand it is important for small satellite laboratories to maintain professional competence and interestingly Waikato Quality Assurance program participants find the program particularly beneficial for this.

Compared to internationally recognised EQA programs, both regional programs differ. The Waikato regional program aligns closely to these programs, with only two significant variables. The frequency of films distributed is higher than the maximum two weekly recommendation, and reports are not confidential. Decreasing the current weekly distribution to two weekly, would assist in the difficulty of finding new blood appearances, and the time consumed by the program, in a busy laboratory. Usage of computer resources would also benefit

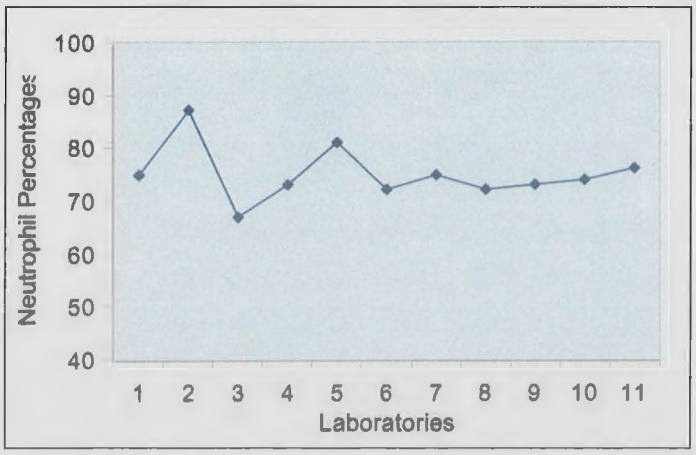

Figure 1. The spread of participant's results of neutrophil percentages from a blood film from a normal pregnant woman distributed in the Waikato regional external quality assurance program. Laboratory one represents the organising laboratory results.

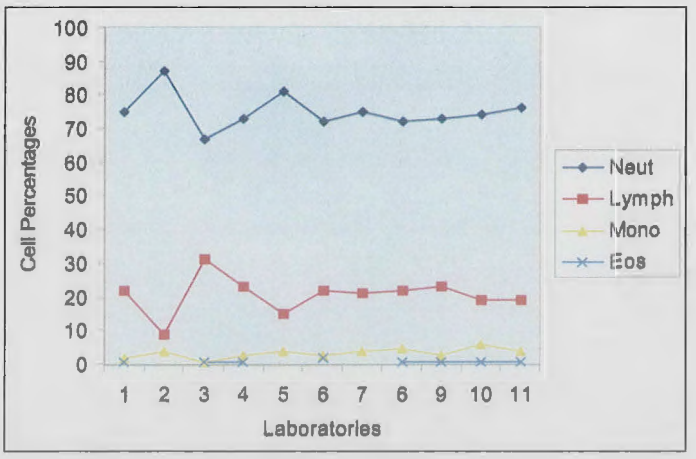

Figure 2. The spread of participant's results of all cell types – neutrophils, lymphocytes, monocytes, eosinophils – from the same quality control sample as in figure 1. Laboratory one represents the organising laboratory results.

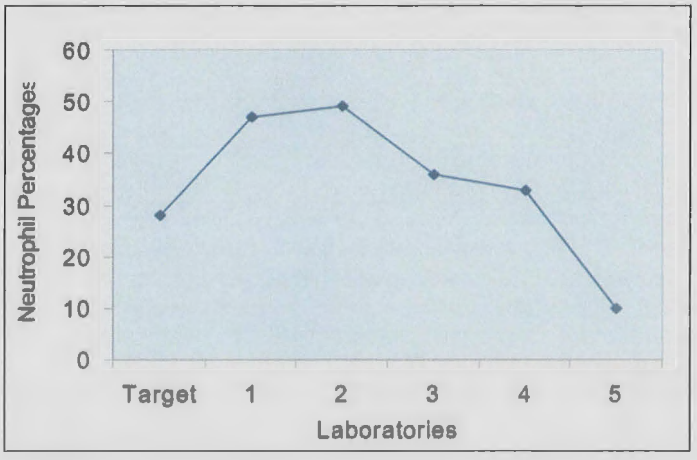

Figure 3. The spread of participant's results of neutrophil percentages from the first survey distributed in the Mid-Western region, from the national external quality assurance program. Laboratory one results, represent the target value calculated from the national organising laboratory.

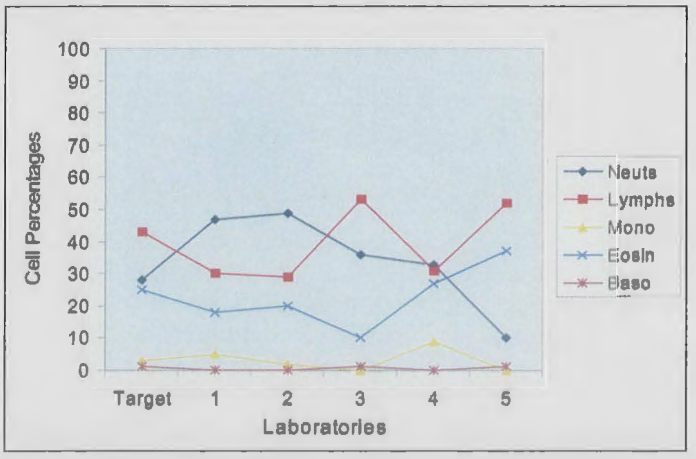

Figure 4. The spread of participant's results of all cell types – neutrophils, lymphocytes, monocytes, eosinophils, basophils – from the quality control sample as in figure 3. Laboratory one results, represent the target value calculated from the national organising laboratory.

program organiser's time. One of the greatest benefits of this program however, is the strong emphasis on it being educational. Satellite laboratories are able to build-up a set of teaching films for training and continual referral and a weekly educational supplement provides professional stimulation where educational resources are not so readily available. At the time of evaluation, a morphology workshop had not been organised for participants, but would provide a complement to the program in the future.

In the Mid-Western regional program, while participation in not voluntary, a level of confidence had already been built with the staff. Physical inspection of the laboratories has been linked with the quality assurance program, and if this were to continue, a strong affirmative relationship between participant laboratories and program organiser's needs to be further established and maintained. Aspects of the program needing review include distribution of unstained blood films, frequency of films distributed and time elapse of reporting. Participants would benefit more, with greater emphasis on education as in the Waikato program (e.g. regular morphology workshops, distribution of an educational supplement and abnormal slides with the EQA). Over time, lack of educational resources would be improved.

Due to time constraints and lack of access to abnormal films, a more comprehensive study of comparative quality between the regions has been limited. A follow-up study after two years with both regions using the same EQA program and with an emphasis on education would be revealing.

This evaluation, and comparative study, has beneficial implications for the future. Evaluation of other laboratory tests could be undertaken to improve total quality management of laboratories, in accordance with international recommendations. Writing custom-designed teaching material and providing necessary resources for the strengthening of

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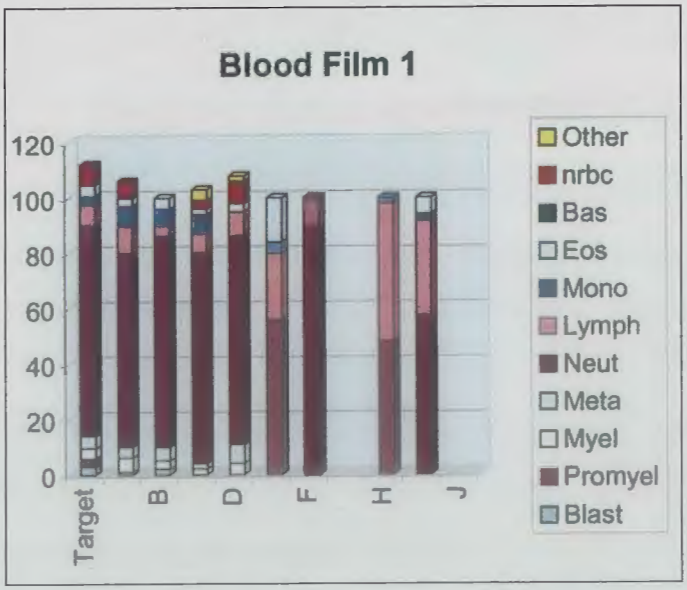

Figure 5

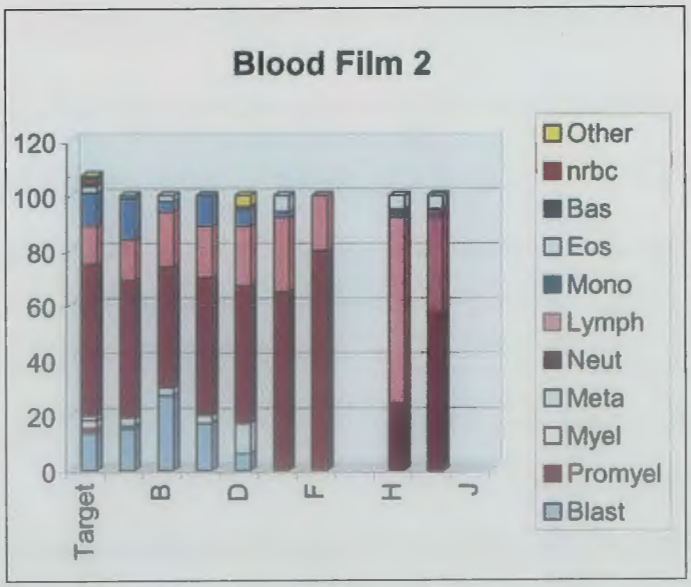

Figure 6

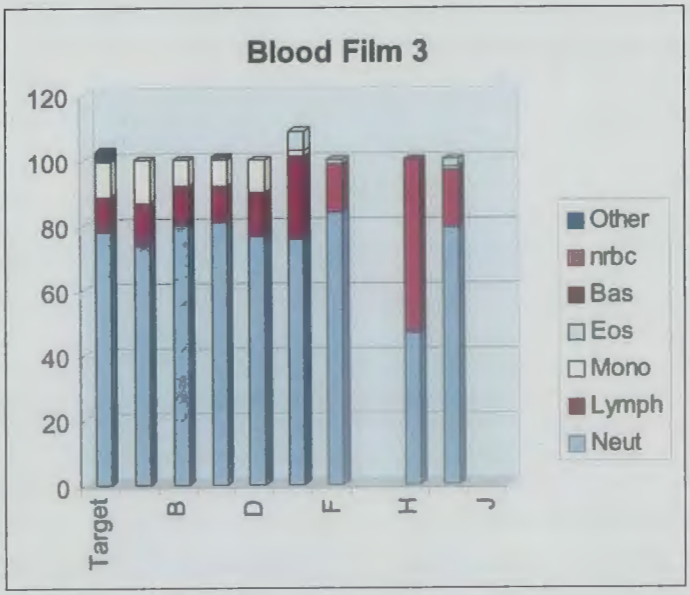

Figure 7

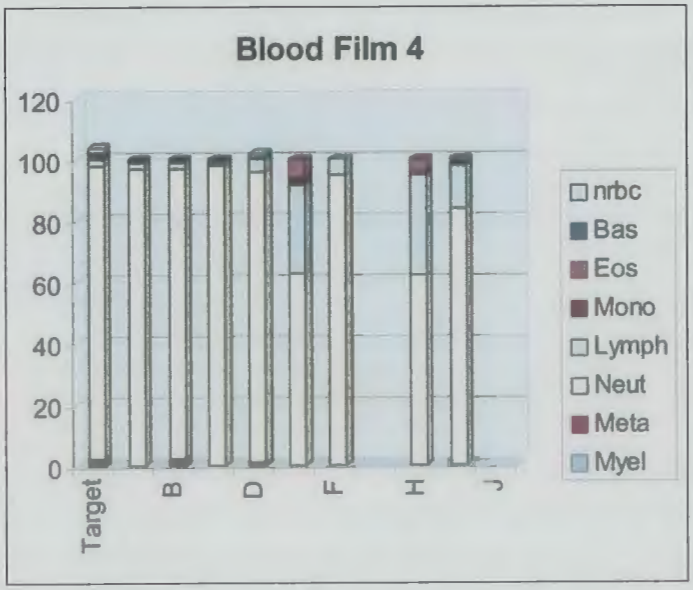

Figure 8

Figure 5-8 A bar graph of QC1-4 leukocyte differential results respectively, from the comparative quality control assessment.

Note:
The laboratories participating in the Waikato and Mid-Western regions external quality assurance programs do not correlate exactly with the laboratories involved in the comparative study, therefore separate numbering for the laboratories is used.

blood film microscopy services in the Mid-Western region, would later lead to providing resources for strengthening of the total laboratory service, throughout Nepal. In New Zealand, the extension of the EQA program to a greater number of satellite laboratories throughout the country would assist in strengthening and maintaining the quality service in laboratories.

Conclusions

The quality of the blood film microscopy service in the Mid-Western region of Nepal lies in contrast to services in the Waikato region and international recommendations, largely due to a lack of resources and training. Greater collaboration between resource poor and rich regions, and countries would assist greatly in bridging the gap.

Acknowledgements

Thanks to Dr Stephen May and Dr Rod MacRorie for their initial inspiration, direction and encouragement. I am appreciative of International Nepal Fellowship, for allowing me study time. Thank you to my good project colleagues, Om Raj Acharya and Shankar Chaudary for helping with translation. Thanks also to the INF Pokhara translation team. To all of the laboratory staff that participated in this study, in both New Zealand and Nepal, I am very grateful. I hope we all benefit. My grateful thanks to Mr Robin Allen and Mrs Sue Webber from the Waikato region, for their support and time in the comparative study and EQA program. Also, for their collaboration, in willingly including Nepal in the Waikato EQA program. Thank you to my friends Craig and Juliette Drown, working in Nepal with us and also to Ash Fitchett of Hawkes Bay Regional Hospital laboratory for proof reading this dissertation.

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Appendix 1

Questionnaire for Government Hospitals

For the purpose of this research, blood film microscopy service is defined as differential count, examination of a blood film specifically for normality or abnormality of white blood cells, red blood cells and platelets. This research does not cover blood film microscopy specifically for malaria or microfilaria.

Region / Country:

Size of Hospital (no. of beds):

No. of Doctors in the hospital:

Consultant Haematologist: Yes No

Population hospital serves:

Does this laboratory provide a blood film microscopy service?
Yes No

How many staff work in the department?

How many staff vacancies are available in the department?

Do you feel the department is overstaffed, understaffed, or staffing is adequate?

How many staff in this laboratory exam blood films?

What qualifications do the staff examining blood films have?

How much training was initially given for blood film examination?

Was the initial training work-site based or from an institution?

Did you feel the initial training was adequate? Did you feel confident to look at blood films after the training?

Do you now feel confident examining blood films?

Describe the staining technique used for blood films in your laboratory.

In which situations would a blood film be looked at? (eg. Ordered by a Doctor, added by lab staff on seeing abnormal Full Blood Count results, etc).

How is it judged whether the staff are competent in their blood film examination? (eg. Competency documents).

Have staff received refresher training? If so, when and for how long?

Is supervision given from outside / inside the laboratory to check work? Explain.

What access do staff have to resources to assist them in blood film examinations (eg. Blood atlases, teaching slides).

How many blood films are examined daily, weekly, monthly, yearly as a laboratory / for staff individually.

How much experience do staff have in blood film examination? (eg. Abnormal's seen).

In looking at a blood film: How do staff evaluate the adequacy in film preparation and staining?

Are Full Blood Count parameters and other laboratory results available when looking at the blood film? If so, which ones?

How is the blood film examined? (eg. Differential count only, comments on cells).

How are blood films reported in the laboratory?

Differential count – percentages or absolute values?

Cell comments (including inclusions)?

Interpretative comments?

Does this laboratory participate in an external or internal blood film Quality Assurance Program? If so, please explain fully.

When abnormal blood films are seen:
Is there another person available to confirm abnormal findings? Who?

Who are the findings referred onto? (eg. Referral laboratory, senior technician / technologist, Doctor, Haematology Consultant, Pathologist).

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Appendix 2

Quality Control Program Evaluation

1. Aims:

2. How is QC material prepared and distributed? To whom?

3. How are the results analysed?

4. What are the beneficial aspects of the program?

5. Is the program achieving was it was set up to achieve? How?

6. What follow-up is there for a laboratory with consistently abnormal results?

7. What are the limitations of the program?

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country, and results may be used to develop microscopy services in either region. I also understand that this information maybe published or presented in a meeting, and I give approval for this.

Appendix 3

Quality Control Program Evaluation for Participants

1. What is the quality of the blood smears on examination?
2. Does your laboratory use the same staining technique as the blood smears received?
3. Is the reporting / evaluation of results from the QC centre adequate for your needs?
4. What do you consider the beneficial aspects of the program and why?
5. What does your laboratory do if QC results are not consistent with results of the other laboratories?
6. What do you consider the limitations of the Program?

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Appendix 4

Blood Film Quality Control Evaluation

Instructions:

Please find enclosed four blood films for microscopic examination. Two blood films are stained and blood parameter / patient information is given reflecting Quality Control in New Zealand. Two blood films are unstained and no blood parameter / patient information is given reflecting Quality Control in Nepal.

On the *stained blood films* perform a differential count, report a comment on the cells and suggest a diagnosis.

On the *unstained blood films* stain the slides and return the slides with your result sheet. For 'QC1' perform a differential count only.

For 'QC4' perform a differential count, report a comment on the cells and suggest a diagnosis.

Ie.

QC1	stain, differential count
QC2	differential count, film comment, diagnosis
QC3	differential count, film comment, diagnosis
QC4	stain, differential count, film comment, diagnosis.

Guidance for doing differential count

1. Count early band forms as metamyelocytes and late band forms as segmented neutrophils.
2. If nucleated red cells are present, count the number per 100 WBC's and report as the number.
3. Report all differentials as percentages.

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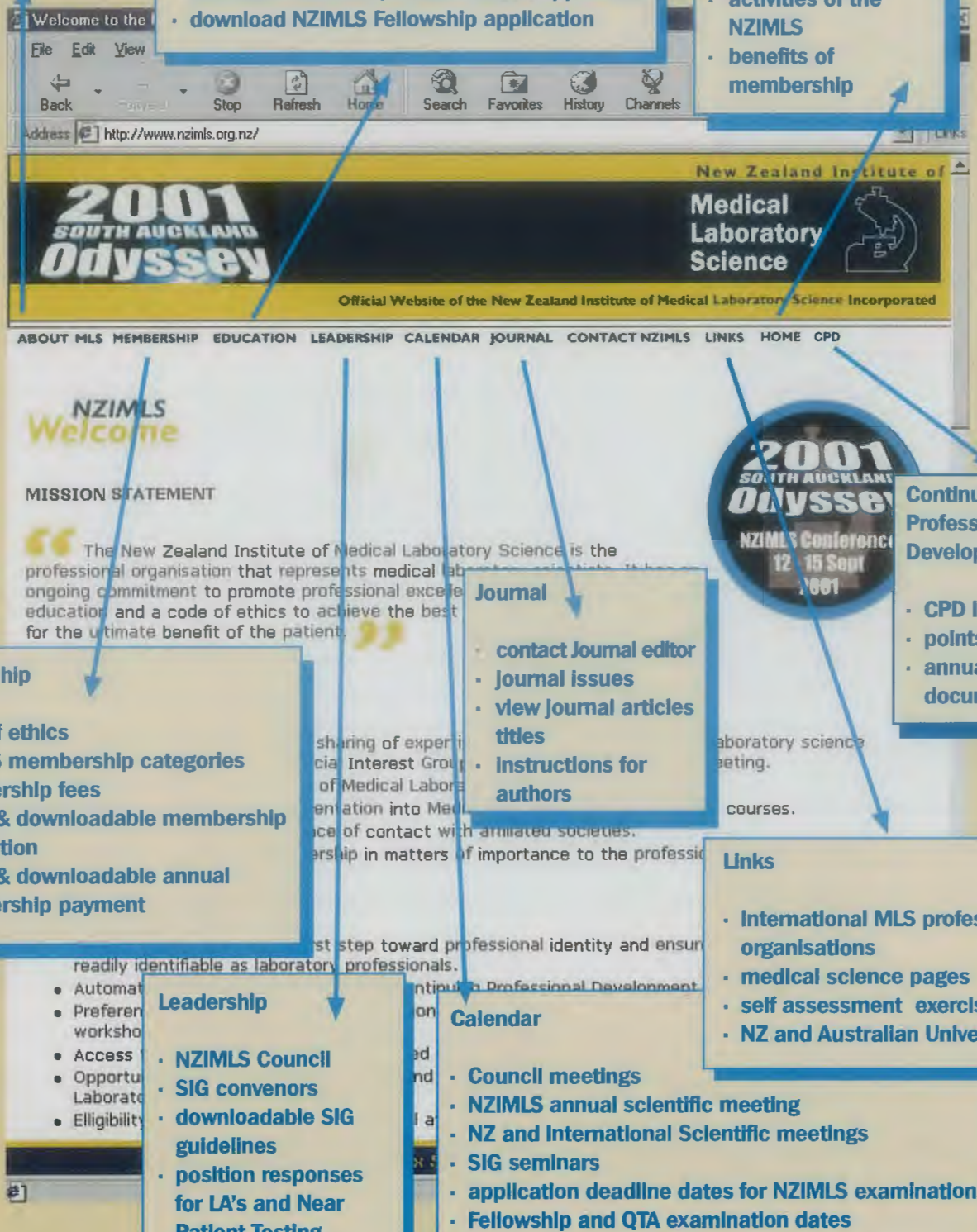

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Letters to the Editor

International Association of Medical Laboratory Technologists membership

Dear Editor,
I am writing to oppose the NZIMLS retaining its membership of the International Association of Medical Laboratory Technologists (IAMLT).

During the 12 years I served as a Council member of the NZIMLS I questioned why the NZIMLS should remain a member of the IAMLT.

My reasons were primarily:

1) That membership of the IAMLT did not really benefit the NZIMLS and that as the NZIMLS was struggling to remain financially viable, the subscription was better spent elsewhere.

2) I was very concerned at the financial state of the IAMLT itself.

The IAMLT has published policy guidelines on Near Patient Testing, the Environment and Educational Requirements for Medical Laboratory Sciences and is writing them on Quality Systems in Medical Laboratory Science and Continuing Professional Development. The NZIMLS has its own policies, which are as good as or better than those of the IAMLT.

Each year member organizations are asked to hold a Biomedical Science day with a set theme. Despite encouragement from the NZIMLS few laboratories in New Zealand have contributed to this idea.

Despite the IAMLT being a Non Governmental Organisation within WHO, it has had little success getting WHO to use the IAMLT to nominate persons for WHO committees or projects.

The IAMLT has established SIGs in the various disciplines and called for nominations for these committees. Only 25 people responded, 8 of whom were from Microbiology.

I concede that there is a benefit to the NZIMLS members through the awards that are offered.

Attempts have been made to help less developed countries through the Development fund which relies on donations for funds, and the Country Project in which the IAMLT acts as "broker" between donor and recipient countries. To my knowledge only one or two projects have been developed, although I accept that these initiatives may take time to expand as member societies are struggling financially.

At the General Assembly of Delegates (GAD) in 1998 the Treasurer warned that the IAMLT would be bankrupt by 2002 unless something was done. This prompted the NZIMLS to seek a legal opinion on the liability of the NZIMLS in the event of financial collapse of the IAMLT. The opinion was "generally of the view that the NZIMLS was not at risk". Since then the only action taken has been to discontinue the Med Tech International Newsletter and replace it with an online newsletter. Few member organisations have bothered to forward news and unfortunately this probably means poorer communication with members, as one has to have access to, or make the effort to go to the website to read news, whereas one is more likely to read printed material received in the hand.

At the General Assembly of Delegates (GAD) in 2000 the Treasurer projected deficits of $40500 for 2001 and $42000 for 2002. A discussion on finances took place at this meeting late in the day, prompting delegates to comment that finance should have been further up the agenda or discussed at the more open forum, the pre GAD meeting. The Chairman replied that "due to the delay in receiving the auditor's report and budget, Council was not completely aware of the actual situation". Does this mean that an organization that knows it is in a precarious financial situation is not keeping a running brief on its finances?

The major expenditure of the IAMLT is the cost of running the office. This is being moved from Sweden to Singapore, not to save money but because the Executive Director is moving there. Council "hopes" that the cost will decrease.

There is a savings account in the UK that is being used up. If this is liquidated it will allow the office to function to 2004 and then the IAMLT will be bankrupt.

The Council of the IAMLT seems unable to make difficult decisions regarding finance, it has not even included a statement regarding finance in its action plan 2000 – 2002.

The NZIMLS had a $36000 deficit last year and has to budget carefully. I do not think the NZIMLS can afford to give $1100 annually to an organization that provides no benefit to it that I can see and that is likely to disappear within the next few years.

Shirley Gainsford
Valley Diagnostic Laboratory, Lower Hutt

Dear Editor,
I am also writing in support of the NZIMLS retaining its membership of the International Association of Medical Laboratory Technologists (IAMLT).

When I read that our Council (NZIMLS) was considering withdrawing from membership of the IAMLT, based on a Motion carried at the last AGM, I was astonished and saddened by this prospect.

In my view this would be a retrograde step in the light of international interchange of medical scientific advancement, also in the significant contribution made by New Zealand colleagues over the years.

I certainly reiterate the comments and history outlined, so well, in the Letter to the Editor by Dennis Reilly (1).

Over the past 40 years of my employment in the health services of this country (medical laboratory technologist/manager and CEO of two divisions of the Cancer Society of NZ) I have enjoyed and appreciated the support and experience shared through interaction with colleagues around the world.

This has occurred by way of journals and study visits to Australia, USA, Hungary, Scandanavia and the UK. Some of these contacts have continued to this day both professionally and at a personal level.

Interestingly, I have found that, invariably, these relationships have been mutually recognised as beneficial.

Consequently, I have always valued our Institute's membership of the IAMLT. Also, I continue to believe that our present day NZ qualified medical laboratory scientists have much to offer in the years ahead at the international level.

G. Frank Lowry, MNZM, MNZIMLS (Non Practicing)

Reference

1. Reilly D. IAMLT membership (Letter). *NZ J Med Lab Science* 2001; 55: 5.

Editor's note.

Membership of the IAMLT by the NZIMLS will be discussed at the 2001 AGM. At the 2000 AGM a motion was carried "That Council refrain from paying the next subscription until this is discussed at the 2001 AGM".

Dennis Reilly, past President of the NZIMLS and past Council Member of the IAMLT, by invitation, presented his views on this topic in the April, 2001 issue of the Journal (1). Shirley Gainsford, past President of the NZIMLS, by invitation, presents her views in this issue of the Journal. As Editor, I invited members of the profession for their views. Only one, Frank Lowrey, has replied. His views are in this issue of the Journal.

Abstracts from articles in the Australian Journal of Medical Science, the official publication of the Australian Institute of Medical Scientists.

Faddy S. Drowning and near-drowning: the physiology and pathology. *Aust J Med Sci* **2001; 22 (1): 4-13.**

Abstract: Drowning is one of the leading causes of premature death in Australia. Hot summer temperatures, a high proportion of the population resident in coastal regions and easy access to beaches, rivers and swimming pools ensure that aquatic activities feature strongly in the leisure time of many Australians. The rate of drowning in Australia is 1.6 deaths per 100,000 population, with 80% being male. Drownings are the most frequent in the 0-5 year age group, followed by the 20-49 year age group. Alcohol is estimated to contribute to as many as 50% of drowning accidents. Developments in the advanced life support capabilities of ambulance officers and public awareness of the importance of CPR education have led to a large number of drowning victims being admitted to the Emergency Department or Intensive Care Unit for treatment. The greatest pathophysiological consequence of near drowning is hypoxaemia, which threatens cerebral, cardiac and major organ function. The drowning incident and several treatments effect the pathophysiological state of the patient and will result in a number of changes to haematological and biochemical markers. Pathology tests help to determine the severity of the injury and guide management in both the acute and long-term stages of treatment. This article reviews the pathophysiology of the drowning process, the associated pathological changes, current recommendations for treatment and the implications of a number of prognostic indicators on the outcome of the resuscitative effort.

Key words: drowning, near-drowning, physiology, pathology, hypoxaemia, acidosis, cardiopulmonary resuscitation, review.

Glasson JH, Lowe AH, Drew DK. Fungal sinus infections: three case studies. *Aust J Med Sci* **2001, 22(2): 80-3.**

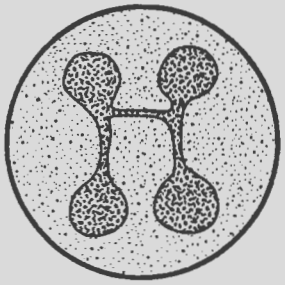
Abstract: Two cases of fungal sinusitis are described in patients who were confirmed immuno-competent, had no history of upper respiratory tract trauma, diabetes or local or systemic corticosteroid therapy. A third case is reported in which a sample of nasal discharge grew Aspergillis flavus but no abnormal findings were reported on extensive examination of the airways. The role of fungi in various respiratory syndromes is reviewed and issues relating to laboratory diagnosis and therapy discussed.

Key words: fungal sinusitis, Aspergillis flavus, Alternaria spp., Bipolaris spp.

Monaghan W. Lemierre's syndrome: clinical presentation and role of the microbiology laboratory. *Aust J Med Sci 2001, 22 (2): 72-9.*

Abstract: Lemierre's syndrome is a human necrobacillosis or more specifically, a postanginal septicaemia. This syndrome is characterized by an initial pharyngotonsillar infection followed by thrombophlebitis of the internal jugular vein (IJV), anaerobic bacteraemia and septic embolisation. Metastatic spread of infection characteristically affects the lungs and joints. Cases occur typically in previously healthy young adults. The syndrome is caused in an overwhelming number of cases by a single anaerobic bacterium, Fusobacterium necrophorum. Lemierre's syndrome was common in the pre-antibiotic era, with the greater majority of cases being fatal. Cases are now rare, and although the mortality rates are comparatively low, reported morbidity rates remain high due to low levels of recognition and delays in diagnosis and optimal therapy. A history of pharyngitis is a key indicator followed several days later by signs of severe sepsis. The microbiology laboratory has a vital role to play in the isolation, accurate identification and prompt reporting of isolation of F. necrophorum. The isolation of this organism from blood cultures is distinctive enough to confirm a presumptive clinical diagnosis or otherwise initiate further investigation with a provisional diagnosis of postanginal septicaemia or Lemierre's syndrome.

Key words: postanginal septicaemia, thrombophlebitis, metastatic foci, Fusobacterium necrophorum.



HSIG Journal Based Learning – Questionnaire

CE Update - Anaemias II "Differential diagnosis of microcytic anaemias"
Judith L. Meredith, MD, Nancy S. Rosenthal, MD.
Laboratory Medicine Vol. 30, No.8, August 1999, p 538 - 542.

Please circle the correct response

1. A decrease in MCHC correlates with the degree of hypochromia in red cells.
TRUE FALSE

2. Chronic inflammation is the second most common cause of microcytic anaemia.
TRUE FALSE

3. Free erythrocyte protoporphyrin is increased in iron deficiency anaemia .
TRUE FALSE

4. Serum transferrin receptor level is elevated in the anaemia of chronic inflammation.
TRUE FALSE

5. The serum transferrin receptor protein is present only on developing red cell precursors.
TRUE FALSE

6. A normal RDW is thought to be more consistent with thalassaemia than with iron deficiency.
TRUE FALSE

7. A cell haemoglobin distribution width (another index of red cell heterogeneity) is the standard deviation of the product of the red cell volume and MCV.
TRUE FALSE

8. The alpha thalassaemia gene is located on chromosome 11.
TRUE FALSE

9. HbH levels in three gene deletional alpha thalassaemia may be decreased in concomitant iron deficiency.
TRUE FALSE

10. The peripheral blood smear in beta thalassaemia trait shows more bizarre features than that in alpha thalassaemia trait.
TRUE FALSE

11. Quantitation of HbA2 is necessary for accurate diagnosis of beta thalassaemia trait.
TRUE FALSE

12. Significant numbers of microcytic, hypochromic red cells are present in 50% of cases of anaemia of chronic inflammation
TRUE FALSE

13. Reticulocytes are increased in proportion to the degree of anaemia in chronic inflammation.
TRUE FALSE

14. Macrophage cytokine secretion makes iron unavailable for haemoglobin synthesis in patients with chronic inflammatory disease.
TRUE FALSE

15. The MCV is useful in distinguishing between the hereditary and acquired forms of sideroblastic anaemias.
TRUE FALSE

16. Iron deficiency may develop following a case of lead poisoning.
TRUE FALSE

17. Basophilic stippling may be a feature of sideroblastic anaemia and beta thalassaemia but not iron deficiency or chronic inflammation.
TRUE FALSE

18. Free erythrocyte protoporphyrin is raised in sideroblastic anaemia.
TRUE FALSE

19. Percentage iron saturation is normal in thalassaemia.
TRUE FALSE

20. Serum iron level is raised in sideroblastic anaemia.
TRUE FALSE

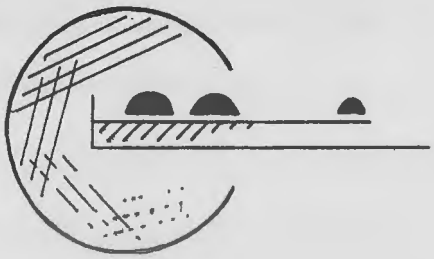
21. Serum transferrin receptor level is raised in iron deficiency anaemia.
TRUE FALSE

Obtain a copy of this article from your medical library, or I would happy to mail a copy to those requesting this option . Contact Jacquie Case , Haematology Dept., Middlemore Hospital, Otahuhu, Auckland . Ph 2760044, extn 8515.
Or e-mail: jcase@middlemore.co.nz.

Answers on page: 65

Microbiology

Special Interest Group



A very successful seminar was held in Hamilton in March. Over 70 people attended the Friday evening sessions on Occult Blood Testing and IANZ registration requirements. Thanks to Sydney Sacks, Phil Barnes, and Linda Manuel for their presentations. It certainly created a lot of discussion, and has led to many labs looking closely at the way they do things.

The Saturday seminar had 140 attending and 17 presentations covering a wide range of topics. Thanks to all of those who presented - it is not easy to face such a large group, but the success of the day is dependent on you. Congratulations to Tina Littlejohn for winning the award for best presentation, and to Chris Jackson for a close second. Thanks also to our supporters (Bayer, Global Science, Ngaio Diagnostics, bio Merieux, IANZ and Health Waikato) and to the team who spent numerous stressful hours in organising the seminar (Catherine Tocker, Kay Stockman, Matt Akehurst, Gail Nielson, Tina Jowsey, Karen Jowsey, Jennie Frost, Carla Shailer, Jan Bird, Chris Pickett, and Steve Soufflot).

Next years seminar will move to the South Island for the first time. It is to be held in Christchurch in May. Julie Vincent (Canterbury Health Laboratories) is the seminar coordinator.

The abstracts from this years seminar are included below.

Steve Soufflot,
MSIG Convenor.

The case of the sore foot. *Joan Byrne - Wellington Hospital*

A 27 year old Samoan office worker who had earlier been diagnosed with SLE suffered a fall and presented to the Emergency Department with a fractured foot. This resulted in ongoing pain, which was treated with a steroid injection. An abscess subsequently developed which on culture grew a pure growth of *Salmonella Typhimurium.* Although stool samples were never received in the laboratory, the source of the organism was thought to be a self-limiting diarrhoeal illness she suffered while holidaying in Samoa. The importance of a correct diagnosis was highlighted by the Infectious Disease physicians who indicated that an abscess would normally have been treated with Penicillin and Flucloxacillin, which in this case would have been inappropriate.

Cryptococcal meningitis - a case study. *Nicola Gelston - Wellington Hospital*

A case study following a young male patient who developed cryptococcal meningitis after a prolonged course of immunosuppressant drugs, including prednisone, for the treatment of sarcoidosis.

The microbiology laboratory examined 7 CSF specimens between October 2000 and March 2001. *Cryptococcus neoformans* was isolated on the second CSF with a positive India Ink stain, encapsulated yeast cells seen in the gram stain, growth on blood agar, chocolate agar and SAB agar, and a cryptococcal antigen titre of 1:16000. Additionally the opening pressure recorded on performing the lumbar puncture was 45cm (normal is <20cm) which is consistent with Cryptococcal meningitis.

The further CSF examinations were done to monitor the patient's progress after a 6 week course of IV amphotericin and oral fluconazole.

Once upon a time. Chris Jackson - *Medlab Hawkes Bay*

Recently a senior lab scientist became ill with pyrexia, nausea and loss of appetite. Preliminary blood tests showed elevated liver enzymes, reduced platelets and a raised ESR. The white blood cell count was 4.3 x109 /L. Investigations for hepatitis and viral disease proved negative.

After 7 days, his condition worsened with increased fever and rigors. Blood cultures were taken and *Salmonella paratyphi* A was isolated. The organism could not be differentiated from an isolate received in a RCPA Quality Assurance Program a month previously. The patient was admitted to hospital and after more blood cultures were taken was treated with IV Ceftriaxone. Just a month later, the same organism was isolated from the blood of a lab scientist working in the Microbiology department of the local hospital. This staff member had a two week history of fever, headache and joint pain.

This case study illustrates the value of taking blood cultures from patients with pyrexia, and reminds us of the risks we all face of contracting laboratory acquired infections while handling virulent organisms.

MRSA screening methods in NZ hospitals. *Henrietta Maguire - Wellington Hospital*

A questionnaire on MRSA screening methods was sent out to 18 hospital laboratories nationwide. All hospitals include a nasal and groin swab in their screen and swabs from ulcer/wound sites. Only a few include invasive devices eg. urinary catheters or faeces (in patients with diarrhoea) in the screen.

In the survey of MRSA screens during February 2001, only 40% of positive patients were detected by MRSA screening. 12% of MRSA screens were detected by enrichment broth only.

As expected, laboratories vary in their methods of processing screening swabs. All labs use an MSA plate containing methicillin/oxacillin as their selective agar. Two labs don't inoculate a primary solid medium, while five labs do not use enrichment broths.

Most labs keep their primary isolation medium up for 48hrs (24-72 hrs). Of those that use enrichment broth, 70% incubate for 24hrs while 30% incubate for 48hrs. Plates sub-cultured from the broths are split almost 50/50 between 24hr and 48hr incubation. TAT for negative screens vary from 24hrs for one lab (no enrichment broth used) to 96 hrs (three labs). Eight labs have a TAT of 48hrs and the remaining six labs put their negative results out after 72hrs.

Published studies suggest that enrichment broths should be used in MRSA screening and that 24hr is sufficient incubation time for the broth. This survey may give labs some food for thought and ideas for adapting methods if required.

Urinalysis beyond 2000. *Carrie Swanson - Medlab Hamilton*

An evaluation of the Sysmex UF100 and UF50 urine cell count analysers in a clinical workplace, and our reasons for utilisation.

The Sysmex UF100 and UF50 are semi-automated urine analysers util-

ising flow cytometry to differentiate and determine concentrations of stained elements by simultaneous measurement of their scatter, fluorescence and impedance. They have preset parameters which flag abnormal results (including glomerular red cells) indicating a manual microscopy is required. The UF100 is able to be interlinked with a dipstick processor, and can analyse 100 samples per hour. The UF50 processes 50 samples per hour, has a superior probe design and an anti-carryover function.

There are no widely recognised UF100 urine reference standards. We obtained presumptive standards after statistical analysis of a two week trial of all urines received, using the Sysmex UF100 and a manual cell count (Kovaslide).

The UF100 was purchased to improve analysis accuracy and assurance, to reduce manual urine microscopy volumes, and to decrease staff costs. It fulfils our expectations in that the results obtained are reproducible and accurate, we have decreased our manual microscopy rate substantially, and hence saved technologist time.

A touch of Danish culture. *Tess Urbanski - Medlab BOP*

SSI enteric media was developed and used by Statens Serum Institute, Copenhagen, Denmark for more than 20 years, for the isolation and identification of enteric pathogens (with the exception of Campylobacter). An evaluation study of this media performed in Tauranga showed a comparison of SSI to XLD/HE and CIN agar for isolation of *Salmonella spp* and *Yersinia enterocolitica*. The study of 1065 faeces specimens tested showed comparable isolation of *Salmonella spp* and *Yersinia enterocolitica,* and showed improved isolation of Yersinia when both SSI and CIN agar were used together. This was because some isolates grew on CIN only and some on SSI only. The cost of the SSI agar plates was comparable to XLD/HE agar plates and the work involved to identify organisms growing was assessed by the number of urea broths, TSI slopes and LIA slopes set up from both media. The number of urea broths set up from SSI was considerably lower than from the XLD/HE - 196 and 302 respectively. This was due to the easy identification of *Proteus spp,* which gave a strong phenylalanine decarboxylase positive reaction on SSI. The number of TSI/LIA slopes used was comparable with 133 and 140 respectively.

Note: Since this study we have trialed a XLD/SSI split plate. Our laboratory has decided to routinely set up an XLD/SSI split plates plus a CIN plate for routine culture of our faeces specimens. We have done this to save cost and time setting up urea broths and to improve the isolation of *Yersinia enterocolitica.*

"Birds of a feather". *Jenny Bennett, Charlotte Kieft, Graeme MacKereth, Carolyn Nicol - ESR*

At the end of 1999, an uncommon phage type of *Salmonella Typhumurium,* STM160, was seen – one case in November and two cases in December, all of them in Canterbury. The only previous case of this phage type occurred in November 1998, also from Canterbury. In 2000 cases continued to occur in Canterbury at the rate of one or two a month, until July, when the first case was seen in Southland. From then on, cases occurred with greater and greater frequency, and became more widespread, until now cases are seen in nearly every health district in New Zealand, although still predominantly in Canterbury.

At the same time as the human cases of STM160, it was noted that sparrows were dying in unusual numbers in the Canterbury region. The first non-human isolate sent to ESR was from a cat, which had no connection to the human cases. This was followed by a post mortem isolate from a cockatoo. The cockatoo had died after eating a sparrow which had flown into its cage at a wildlife park in Christchurch. The owner of the wildlife park was concerned at the possible risk to native bird-life from contact with infected sparrows, and contacted the Department of Conservation which, together with the Massey University and the National Centre for Disease Investigation, began a national study of bird deaths.

It was found that STM160 was present in many different species of birds, although sparrows predominated. Bird deaths were recorded throughout New Zealand. STM 160 has now also been isolated from dogs, cattle, horses, rabbits, goats, poultry and deer.

Macrorestriction DNA analysis using pulsed-field gel electrophoresis of Xba1 digests showed that all isolates since 1999, both human and non-human, belong to the same clone.

Reports of infection with STM160 from countries other than New Zealand are rare. It is still not known how this unusual phage type was introduced into New Zealand.

Out on a LIM. *Tina Littlejohn - Medlab Central, Palmerston North.*

In conjunction with our Infectious Diseases Physician implementing a prevention strategy towards perinatal Group B Streptococcal (GBS) disease at MidCentral Health, our laboratory included a LIM broth into our genital swab culture regime. I conducted a study of the increased isolation rate of Group B Streptococcus after the addition of the LIM broth.

LIM broth is Todd-Hewitt containing colistin and naladixic acid (Oxoid-Global Science).

A vaginal and rectal swab from pregnant women to be taken at 35-37 weeks gestation was recommended. With this timing there is a positive predictive value of 85% and negative predictive value of 97%. The USCDC morbidity and mortality weekly report guidelines suggested that by adding a selective media isolation rates should increase by up to 50%.

By decreasing the use of prophylactic antibiotics on GBS carrier women during labour the adverse effects are reduced. For example, with random administration on a population of four million deliveries annually you would expect ten deaths per year from anaphylaxis, assuming a 25% colonisation rate. In most populations studied 10-30% of pregnant women are colonised with GBS. Of all infants born to these colonised mothers approximately 1-2% will develop early onset invasive disease.

In my study I collected statistics on 1200 samples over three months. From 1200 specimens we had 273 (23%) positive for GBS. Out of the 273 positives, 192 were positive from the LIM broth only. Therefore by including a LIM broth to our routine culture protocol we have increased our isolation rate of GBS by 70%.

A LIM broth is now part of our routine culture regime and we are continuing to encourage the correct timing of samples and a specimen from both sites.

Selexid. *Joanna Stewart - Diagnostic Medlab*

Selexid is the trade name given to the pro-drug pivmecillinam. It is an orally administered b-lactam antibiotic for empirical use in treating acute urinary tract infections. The drug has a narrow spectrum of activity, being highly specific against Enterobacteriaceae, particularly *E.coli* the most common urinary isolate.

Increasing resistance against regularly used antibiotics may warrant the introduction of another drug that has shown to not induce resistance. By continuing to use standard antibiotics there is a risk of resistance levels reaching, in some cases, more than 10%. This could lead to the drugs becoming ineffectual in treating more serious diseases.

In the 20 year history of pivmecillinam use in Scandinavia there has been no increasing resistance to this antibiotic. It does not share general cross-resistance with other penicillins. Susceptibility studies at DML have shown a 4% resistance level of *E. coli* to mecillinam. This is

similar to the resistance levels found in Scandinavia.

Apart from the normal allergy risk as with other penicillins, mecillinam has few side effects. This is largely due to rapid absorption in the gut, which reduces intestinal concentrations, and its narrow spectrum of activity.

This antibiotic is being re-introduced to the New Zealand market.

A case of having gone to the dogs. *Gail Nielson - Health Waikato, Hamilton*

Hydatid Disease is thought to be extremely rare in New Zealand today, although it can be found worldwide in mainly rural areas. It is a tissue infection caused by the larval stages of the *E. granulosis* tapeworm. It is a notifiable disease with an average of five new cases reported each year. Humans can be an accidental intermediate host at the egg stage after contact with an infected dog. Having been swallowed, the egg hatches in the small bowel and releases an oncosphere. This penetrates the intestinal wall, migrates through the bloodstream into various organs, and develops into cysts. Because hydatids can be dormant in the human body for 10-20 years, diagnosis is sometimes difficult with symptoms mimicking other diseases. These are abdominal pain in upper right quadrant, jaundice, bloody sputum and/or chest pain, fever, fatigue, or joint pain. Diagnosis can be made by a physical exam, X-rays, CT scans, aspiration of cyst fluid for microbiology, and blood tests (hydatid complement fixation, hydatid haemagglutination and latex agglutination - all of which can be all negative in the presence of the disease).

In July 2000 a liver aspirate, from an 83 year old man , was found to contain hydatid protoscolices and hooklets in the gram stain. These were confirmed by the trichrome stain. Treatment can involve long term chemotherapy (at least 12 months), surgery and PAIR (Percutaneous hydatid cyst Aspiration, Instillation of a scolicide, and Reaspiration). Outcome depends on individual cases and follow-up every 1-2 years is recommended in the WHO guidelines.

Baa baa black sheep. *Bruce Dove - Diagnostic Medlab*

Anthrax is an acute infectious disease caused by the spore-forming bacterium *Bacillus anthracis.* It is primarily a disease of domesticated and wild animals, particularly herbivores, with humans becoming infected incidentally when brought into contact with diseased animals or contaminated animal products.

Cutaneous anthrax is the most common form of the disease (approx. 95%). Usually acquired via injured skin, the infection leads to the formation of a painless ulcer with a black necrotic center, the characteristic eschar. The infection may disseminate and 20% of untreated cases will result in death.

The bacterium readily grows on routine laboratory media such as blood agar after 24hrs aerobic incubation at 370C. On blood agar large non-, or weakly haemolytic colonies form, which on aging develop the characteristic "Medusa Head" appearance. It is invariably sensitive to penicillin.

An arm swab from a person who had a lesion resembling that caused by *Bacillus anthracis* with the clinical particulars "?Anthrax" was received for investigation at our laboratory. Fortunately, instead of growing this bacterium, the more commonly encountered organism *Pseudomonas aeruginosa* was isolated. This can cause a lesion characterised by necrosis and surrounding erythema (somewhat similar to anthrax) and is known as ecthyma gangrenosum.

VRE : putting theory to practice. *Jane Hunter - Labplus Auckland*

Thirteen cases of VRE have been isolated in New Zealand since 1996. For infection control purposes it is important to rapidly and reliably identify Vancomycin Resistant Enterococci to species level.

A Vancomycin resistant *Enterococcus* species was isolated from an elderly CAPD patient. The organism was identified as *E. gallinarum* when tested by API Rapid ID 32 Strep. BBL GP Crystal, methyl D-alpha-glucopyranoside (MDG or MADGE) broth, and motility testing identified the Enterococcus as *E. faecium.*

The organism was confirmed by ESR as *E faecium* using PCR techniques. ESR recommends the use of additional tests, such as MDG (or MADGE) Broth, 6.5% NaCl and motility testing to confirm the identification of *Enterococcus faecium*

NICE Abstracts

What does my signature mean? *Roger Austin, NZBS- Auckland*
We sign our names to all sorts of documents. Do we really understand what our signature means? What are we agreeing to, what are we taking responsibility for? Read the implied fine print.

The MUD (Matched Unrelated Donors) pool .NZ Bone Marrow Donor Registry. *Raewyn Fisher, Auckland*
Why do we need a registry of Maori and Pacific Island donors? What have we achieved so far?

A case history of neonatal alloimmune thrombocytopenia and its association with HLA". *Gill Morley, Hastings Hospital*

Thrombotic thrombocytopenic purpura: case presentation and review of the literature. *Geoff Herd , Whangarei Hospital*
Thrombotic Thrombocytopenia Purpura (TTP) is an uncommon multisystem disorder which comprises microangiopathic haemolytic anaemia, thrombocytopenia and fever with a variable degree of renal insufficiency and fluctuating neurological abnormalities.

TTP occurs in a heterogeneous group of patients with an increased incidence in association with pregnancy, autoimmune disorders, infection, malignancy, drug exposure, bone marrow transplantation and HIV-1 infection.

Recent studies suggest that endothelial cell damage caused by unknown plasma factor(s) may lead to the release of abnormally large von Willebrand Factor multimers which promote the deposition of platelet microthrombi.

Exchange transfusion with plasma or plasma cryosupernatant is the mainstay of treatment along with corticosteroids, anti-platelet agents and vincristine.

This paper includes an illustrative case presentation and review of the current literature on this obscure but fascinating disease.

The friendly islands : an overview on blood donation. *Tala Teu, Middlemore Hospital*
A summary of the service provided by the Transfusion Laboratory in Tonga, mainly on Tongatapu island. The discussion will focus mainly on the whole process of obtaining blood from a donor up to the stage where the unit of blood is compatible and safe for transfusion to a patient

Fossill fuel. *Adeline Tjia, Middlemore Hospital*
Middlemore Hospital is a major burns unit for New Zealand. Part of the treatment of these patients is the transfusion of blood and blood products. Mostly, this causes few problems for Blood Bank, occasionally as in this case problems arise. My presentation will discuss this case.

The other side of the fence. *Sue Melvin, Invercargil*
A case study on a transfusion medicine scientist who recently experienced life on the other side of the fence. Patient X is Charge of a Transfusion Medicine Department and has recently delivered a beautiful baby boy. Her pregnancy was uneventful, but she is RH negative, so had routine blood tests, and requested her husband be grouped.

Delivery went well, but in 3 weeks later haemorrhaged, requiring hospitalising and a blood transfusion. Discussion on transfusion in this population group would be welcome.

Why don't we trust the couriers? *Graeme Bennett, Medlab Timaru*
A look at the transformation of the blood supply, from Timaru Hospital trying to provide all its own blood to the new, flash, efficient NZBS.

A number of interesting and praise worthy observations are able to be made by an "outsider" in such areas as wastage, supply, testing and couriers.

What is going on here! *Iris Lee, NZBS- Wellington*
An investigation into the continued inability to Leucodeplete the platelet apheresis collections from one particular donor.

ABO blood groups and transplantation. *Kim Gribben, NZBS-Auckland*
Does the ABO blood group compatibility between donor and recipient organ transplants cause a significant difference in the outcome of transplants? A review of the various medical studies performed.

Blood donors. *Peter Webster, Nelson*
Are we looking after this very valuable resource the way we should, or are they just being treated as the supplier of a product we need.

"...And on that farm he had A, B, DEA, M, R, S, T, Qa ..." *Simon Benson, NZBS- Auckland*
"...Old Macdonald has a farm, and on that farm he has..." a wide variety of animals in some of which have been described blood group systems with complexities to rival those in humans.

From 3 simple groups in cats to over 30 in horses, there is more to our four-legged friends than meets the eye.

A is for apple sauce, B is for boysenberries... *Sue Steele, Medlab Tauranga*
Research shows that there is a blood-type profile for many aspects of our lives. According to blood type lifestyle can be adapted to deal with stress, maximise your health and overcome disease.

Are you my mother? *Grant Bush , Medlab Tauranga*
I am a group AB, you are a group O...

A changing ABO population? *Leigh Mosen, NZBS- Auckland*
A look at the ratios of blood groups at Auckland and Starship Hospitals. With our changing ethnic population, are our blood groups changing too?

Changes. *Tracy Inder, NZBS- Otago*
"Even if you are on the right track, if you just sit there you will get run over". Profound words in this ever-changing world we live in. I have observed a number of changes in my Transfusion Medicine lifetime (all 2 years of it!) and am only too well aware that if we do not have the ability and willingness to adapt to such changes we will be left behind. Thus affirming the importance of continuing education and support of staff.

Case study of an AML patient multi-transfused. *Natalie Fletcher, Tokoroa Hospital*
A male patient was diagnosed with AML aged 17yrs. He was multi-transfused with red cells, platelets and plasma to treat anaemia caused by chemotherapy. He has had two bone marrow transplants and is currently in remission again but recently diagnosed with haemachromatosis, one unit being venesectioned fortnightly! What's up with that!

How much is enough? *Ray Scott, NZBS*
The demand for fractionated blood products must be supported by an appropriate and timely supply of plasma. This presentation will identify the factors affecting the supply of plasma in New Zealand, and the approach taken to determine an acceptable balance between plasma supply, fractionated product stock levels and clinical demand.

Leucodepletion. *Amanda Hayward, NZBS- Waikato*
An overview of what we, in Waikato, have been up to for the last 2 months.

CompoXmas. *Andrew Mills, NZBS- Waikato*
All we want for Xmas is.....!

CMV in the age of leucodepletion. *Brydon Broadley, NZBS- Christchurch*
This is a brief presentation discussing CMV and the transfusion of blood and blood products. Will the introduction of leukodepletion alter the management of CMV in New Zealand?

Serious Hazard of Intra-Operative Transfusion (SHIT). *Alan Neal, Rotorua Hospital*
Serious adverse reactions to Blood or Blood products occur infrequently (thankfully), however like any good 'boy scout' we should "Be Prepared" to quickly respond to these events. A rare complication to a Blood Transfusion shall be presented, which with the help of all the 'Transfusion team' resulted in a successful outcome.

"Kell or not to Kell". *Diane Matheson, Rotorua Hospital*
Clinically significant antibodies are commonly stimulated by Blood transfusion. Should we consider women of 'Child bearing age' as special.

Red, white and blue. *Charlotte Sankey, Rotorua Hospital*
These colours all have something in common, other than featuring on many of the world's flags. They are all involved in a severe & potentially life threatening complication of transfusion. This link will be explained through a case study and a brief overview of this type of reaction.

NAT testing. *Heather Richards, NZBS- Auckland*
Later in the year a new technology in Blood screening called NAT (Nucleic Acid Testing) will be introduced by the New Zealand Blood Service to enhance the safety of the blood supply. With every new process ways of doing things must be different. What is NAT? How will the way we do things be different?

Hereditary haemachromatosis. *Claire Lamont, NZBS- Auckland*
People from Irish/Celtic decent are more inclined to suffer from this disease affecting the liver and believe it or not, it has little to do with their alcohol consumption.

Leucodepletion: pooled buffy coat platelets. *Donna Beswick, NZBS- Waikato*
With the introduction of Leucodepletion a new method of platelet preparation has been put into practice at NZBS Waikato...- ...an overview of this method.

Poster presentation. *Scott Reilly, NZBS- Wellington*
My poster will present an information based flow-chart for the process and diagnosis of HDN.

Ozone and transfusion science. *Colleen Behr, NZBS- Auckland*
Ozone is believed to act as a selective and rapid oxidizer of viruses (HIV, Herpes simplex and zoster, cytomegalovirus, Epstein-Barr, myxoviruses and retroviruses), bacteria (coliform and staphylococcus) and virally infected cell membranes. Although used on nearly 500,000 patients in Western Europe since the 1960's, the therapeutic use of medical ozone is largely unknown. Sometimes called bio-oxidative therapy or auto-hemotherapy, mixtures of ozone and oxygen are mixed with blood before re-infusion by clinicians.
Could ozone treated blood donations be the answer to finally eliminating transfusion-transmitted disease?

HDN and antibody identification –a discomfort zone. *Lorna Wall, NZBS- Auckland*
Nobody likes to feel that what they thought was a simple case of antibody identification was not. While one thinks they are in the comfort zone and it becomes the dis-comfort zone . What next?

Southerner derailed. *Jacinta Payne, NZBS- Otago*
On the 8th January, 2001 the Dunedin transfusion service received a seam team message " Emergency A&E, eta not sure, Southerner derailed north Oamaru, DISASTER PLAN " A personal account and review of our efforts to effectively deal with this situation.

Abbott Prism™ - infectious serology screening in half the time. *Vanessa Moriarty, NZBS- Christchurch*
The introduction of the Abbott Prism™ analyser into the New Zealand Blood Service Donor Accreditation Laboratory in Christchurch is set to have an effect on work-flows in this department. Validation of the Prism™ is currently underway in both Auckland and Christchurch Laboratories, working towards a standardised accreditation scheme throughout New Zealand. The different methodology employed by this analyser, in comparison to the screening methods in place in Christchurch, indicate that infectious serology screening will be more efficient, with the added benefits of increased sensitivity and specificity. There is a potential for an increased throughput and faster output of accredited units at New Zealand Blood Service Christchurch.

Cold Hearted. *Astrid van den Berg , NZBS- Waikato*
A case study on the problems and solutions associated with cardiac surgery on a patient with cold agglutinin disease.

Quatro™ SP400 Blood Grouping analyser. *Jeanette Jordan, NZBS- Christchurch*
General overview of the capabilities of the Quatro™ SP400 and the

effect it will have on the workflow and the lives of the donor accreditation staff in Christchurch NZBS. Where we are currently in validation and what problems we are facing.

Conditioned cryo. *Christine Van Tilburg, NZBS- Auckland*
It's a Rachelle Hunter product!

The supply of blood for transfusion to a forward surgical team: the New Zealand experience. *Malcolm Rees, Royal New Zealand Army Medical Corps, 2nd Field Hospital, Linton Military Camp, Linton*
The supply of blood for transfusion, in a safe and timely manner is one of the critical medical/ logistical constraints for a forward surgical team. This presentation describes the standards, equipment and processes used to transport blood to the New Zealand Forward Surgical Team (FST) in Suai, East Timor.

The supply and equipment validation systems employed were developed by a co-operative approach between the New Zealand Army and the New Zealand Blood Service (NZBS). These systems will be discussed in the course of the presentation.

Review of white cell counting. *Tony Mace, Pathlab Hamiltion*
This paper will look at the development of white cell counting to present day.

A case study. *Bev Nickle, Pathlab Hamilton*
A case study that may provoke discussion on ethical and moral issues.

"Statistical figures on red cell usage for Christchurch blood bank and the local hospitals". *Gemma Fong, NZBS- Christchurch*
Using graphical figures, as management tools, to illustrate the seasonal changes on red cells usage.

Do u or do u not? *Tony Morgan, Hastings Hospital*
A review of Weak D Testing and how we utilize the results obtained.

D.A.R.E. *Sheryl Khull, NZBS- Manawatu*
Drugs, Antibodies, Reactions, and Explanations. A case study of a classic case of drug-induced auto-immune haemolytic anaemia.

So you've got new equipment... and when did you say you were going to use it? Tomorrow!!! *Suzanne Williams, NZBS- Christchurch*
A description of the requirements and work involved in validating new equipment before it can be placed into routine use.

Prions- the last frontier. *Ingrid Christiaans, NZBS- Otago*
What are they? How do they work? Will we ever be free of them?

The advantages of being Le(a+b). *Bevan Lockwood*
The association of being Leb+ allowing a receptor for Helicobacter pylori infection in the stomach and duodenum.

Shift Work, the 24-Hour Society. *Darryn Knight, NZBS- Auckland*
In the 24 hour society people engage in rotating night shift work, the circadian rhythms are unable to quickly adapt to a rapidly changing activity schedule. This results in de-synchronosis of many physiologic systems, including those with circadian timing. Diet, sleep hygiene, cardiovascular health, and the need to address social and domestic tensions will be discussed.

AAA case with a twist. *Nadene Hoskins and Stacey Fox, LabCare New Plymouth*
We received a crossmatch request for a 75-year-old female who was admitted to A&E with query AAA. A check of our abnormal file showed she was O Rh(D) negative with anti-D, we had only 15 O Rh(D) negative units in the bank and the nearest center to get more than 3 hours away. However the outcome was not what we expected.

Case study of an AAA patient. *Tirath Lakshman, NZBS- Wellington*
Case study of a 78-year old male with a query AAA with a request for six units of blood urgently from Accident and Emergency.

HSIG journal based learning questionnaire: "Differential diagnosis of microcytic anaemias"
Laboratory Medicine, Vol. 30, No.8, Aug. 1999, p 538-542.

Answers

1. True
2. False
3. True
4. False
5. False
6. True
7. False
8. False
9. True
10. True
11. True
12. False
13. False
14. True
15. True
16. True
17. True
18. False
19. False
20. False
21. True

Advertisers in this issue

Employment agencies

Obituary

John Case

It is thirty years since John Case left New Zealand for a new post in Sydney, but his name will still be recalled by older members for his keen interest and support for the NZIMLT. He undertook many tasks to this end. He was editor of the New Zealand Journal of Medical Laboratory Technology and Newsletter for seven yeas from 1963-1970 and transformed the Journal into a publication of merit with an international circulation. He was a prominent member of the staff side which in those days negotiated our salaries and conditions of service with the State Services Commission and was a fearless negotiator with a detailed knowledge of the requisite regulation who saw that justice was done. He was also an examiner and an excellent speaker but his influence transcended all these roles.

From a personal point of view I shall greatly miss the correspondence we have kept up over the years particularly the twenty page Christmas letters.

Derek Ford, who worked with John in Dunedin, has written an obituary for his Australian colleagues, which succinctly records the details of John's career, and has kindly given permission to reproduce it herewith.

Bob Allan

Immunohaematology lost a renowned medical scientist with the recent death of John Case in Houston. John inspired many medical scientists to specialise in blood group serology by his very individual form of enthusiasm and dedication. I was just one of those who John inspired and he was my mentor, teacher and friend throughout my entire career.

John commenced his medical scientist training at the Public Health Laboratory in Poole, Dorset. Following his two years in the army, from 1945, John held posts in Whipps Cross Hospital and the South London Blood Transfusion Service (where Laurie Marsh was also employed), before emigrating to New Zealand in 1959 to take the position as Chief Technologist in Haematology and Blood Transfusion at the Otago Medical school. It was in 1961 that I first met John, when I joined him as Senior Technologist; from that point on, John was the leading influence in my career as he encouraged me to specialise in immunohaematology.

In 1971, John moved from Dunedin to Melbourne, Australia, to take the post of Consultant Serologist at the Commonwealth Serum Laboratories (CSL) and to head the National Blood Group Reference Laboratory. Whilst there, he quickly expanded and improved the range of blood group reagents that were produced by CSL and was a leading force in assisting the growth of the blood transfusion service in Malaysia. Within only a few years, John was well known throughout the blood banking international scene and, only five years after joining CSL, he was headhunted by Gamma Biologicals of Houston, Texas to become Vice-President of Regulatory affairs. From that time, John played a leading role in the "English Mafia" of USA blood banking and became a well-respected teacher, adviser and consultant. In 1990, John was honoured by the AABB by being awarded the Ivor Dunsford Prize for that year and in 1997 he was given the Ruth Sanger Oration Award by the ASBT. John's forthright manner sometimes rankled a few feathers, but he never hesitated to openly state his opinion – which was always based on his extraordinary theoretical and practical knowledge. Over the last few years of his career, John's exchanges with John Judd in the discussion pages of the AABB web site kept many amused, but never failed to educate at the same time.

John's love for immunohaematology can be gauged by the fact that he did not retire at 65, but continued until his 73rd year; only two years before his untimely death following a stroke. John's sudden passing came as a shock to many immunohaematologists, both medical and scientific, throughout the world and leaves a gap that can never be filled. He is survived by his wife, Ellen and children, Edward, Jackie and Adrienne, and granddaughters Kelsey and Landa, to whom we offer our sincere condolences.

Derek Ford

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