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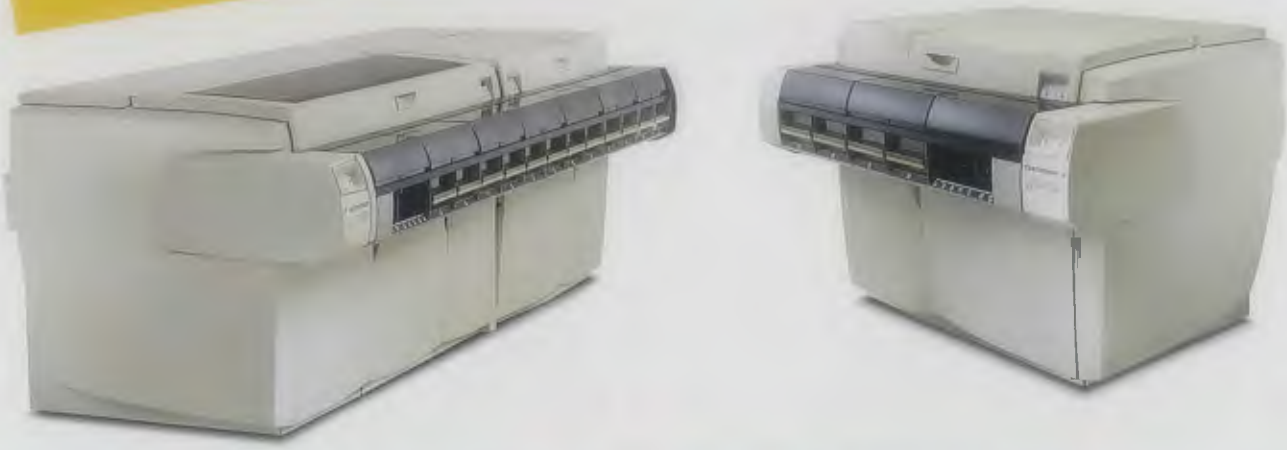

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Editorial

Original articles

Regular features

Editorial

CPD programme update

Jillian Broadbent, FNZIMLS
Canterbury Health Laboratories, Christchurch

CPD coordinator

The beginning of February saw me taking up my appointment as the Coordinator for the NZIMLS CPD Programme. Many of you may already know me, but for those who don't, I shall give you a brief overview of my career to date.

Jillian Broadbent (nee Wilson) - I trained at Auckland Hospital Laboratory and completed a Part 2 and Part 3 in Clinical Biochemistry. Having trained the "old fashioned" way means that I have spent time in most disciplines of Medical Laboratory Science. I spent some time working at Greenlane Hospital laboratory where I was Technologist in Charge of the Protein and Lipid Laboratory before moving to Christchurch to work as the Diagnostic Sales Representative for Boehringer Mannheim (now Roche Diagnostics). My portfolio included Medical Laboratory Diagnostic products for the Clinical Biochemistry department including reagents and major instrumentation, Research Biochemicals especially those for the Molecular Biology sector, Point of Care instruments, Food Analysis kits and Customer Training.

Family considerations kept me temporarily out of the work force for a while, but I now work part-time in the Steroid and Immunobiochemistry laboratory at Canterbury Health Laboratories. In 2003 I was awarded my Fellowship of the NZIMLS for my treatise on Salivary Testosterone.

My experience on the bench, my part-time position in the laboratory and my sales and marketing skills will hopefully make me a capable and understanding person to coordinate and facilitate the implementation of the CPD Programme into participating NZ Medical Laboratories. (Yes, I also have to earn CPD points!)

CPD enrolments

These are continuing to be processed with many laboratories having taken advantage of the discount offered for group membership. Individual email addresses are necessary for access to CPD records and all CPD activities for each participant can be logged through the NZIMLS website.

Regulations now require all Medical Laboratory Scientists to participate in a competency programme and Annual Practising Certificates will not be issued by the Medical Laboratory Science Board (MLSB) this year unless you are enrolled in a CPD programme.

Activities

The NZIMLS council will meet with the MLSB in Wellington, areas of interest to include MLS registration and CPD programme review.

Negotiations are under way for the audit process of the CPD programme. This will be an annual requirement and approximately 10% of participants will be audited. It is important to keep records of all activities you have claimed points for.

SIG Convenors are currently working on programmes for the Structured Reading section of the CPD programme. As soon as these are available, they will be electronically mailed to all participants and also posted on the NZIMLS website.

A section for Frequently Asked Questions (FAQ) is currently under development on the NZIMLS website and all suggestions and feedback are welcome from participants.

A web-based process for application for approval of meetings as CPD approved activities and guidelines for submission of scientific programmes for CPD points approval will be developed.

Web based learning programmes are being researched so that staff working part-time or permanent shifts will not be disadvantaged and Continuing Education will not be inaccesible to them.

I hope to visit as many Medical Laboratories as possible during this year to discuss the programme and to understand any issues staff may have with managing and accruing their points. I also hope to recruit CPD officers in most of the laboratories so there is someone on-site to answer any questions or clarify any matters you may have difficulty with.

I look forward to any communication you may have on the programme and all questions and comments are to be encouraged and appreciated. The introduction of this programme will hopefully strengthen our profession, enhance our continuing education and ultimately benefit and protect the public of New Zealand.

Contact details: Jillian Broadbent
Email: cpd@nzimls.org.nz
Phone: +64 3 343 5177
Mobile: 027 242 4712

Vibrio cholerae in New Zealand

Jenny Bennett, FNZIMLS, Scientist
Enteric Reference Laboratory, ESR Kenepuru Science Centre, Porirua

Abstract

Toxigenic strains of Vibrio cholerae, although not endemic in New Zealand, are isolated occasionally from recent overseas travellers. Isolates should be confirmed biochemically and serologically and the presence of cholera toxin genes established by a reference laboratory, as non-toxigenic strains are found here in the summer months. Serotypes O1 and O139 are the causative agents of epidemic cholera, but the potential exists for other serotypes to become of public health significance because the production of cholera toxin is phage-encoded. It is also likely that the receptor enabling uptake of the cholera toxin phage and attachment of the organisms to the gut wall is phage-encoded, and thus both virulence factors are potentially transferable to previously non-pathogenic vibrios. Toxigenic strains of Vibrio cholerae are notifiable in New Zealand.

Key words: Vibrio cholerae, toxigenic strains, serotype, New Zealand

Introduction

Modern air travel has changed our expectations of likely gastrointestinal pathogens. An organism that has been ingested on one side of the world can reappear in a faecal specimen taken on the other side of the world 12 hours later, which can be less than the incubation period of some diseases. A history of recent overseas travel is hugely relevant to the microbiologist trying to decide on which pathogens are likely to be isolated. Sadly, these details are often not provided and workers have to either guess (patient name likely to indicate travel...? patient age group likely to indicate travel...? medical practice/doctor/clinic referring specimen specialises in travel medicine...? sample looks like it contains 'something really nasty'...?) or culture the sample on every available medium to ensure nothing is missed.

This is not a new situation. In November 1972, a 65-year old man died in Lower Hutt from cholera, acquired following consumption of an aircraft-meal taken on board at Bahrein (1,2). Three cases of cholera and three carriers were identified in this outbreak. No secondary transmission was detected, and the two cases surviving the infection were not seriously ill, but this scenario illustrates the need to be aware that a faecal sample may contain a pathogen not endemic in New Zealand.

The Enteric Reference Laboratory at ESR generally confirms one or two cases of toxigenic Vibrio cholerae a year, all acquired from overseas. Non-toxigenic strains are endemic in the warmer months and are usually associated with bathing, especially in the Eastern Bay of Plenty. These non-toxigenic strains are reported as V cholerae non-O1, non-O139 and were previously known as non-agglutinating vibrios or NAGs. In June 2004, two unconnected cases of toxigenic V cholerae infection were confirmed in one week, prompting this technical report.

Microbiology

V cholerae is a gram negative, oxidase positive, fermentative bacillus that exhibits characteristic darting motility. It is readily grown on thiosulphate citrate bile sucrose agar (TCBS) agar, on which colonies are luxuriant and yellow in colour, due to the fermentation of sucrose. Initial pre-enrichment in alkaline peptone water (pH 8) can be used with subculture to TCBS after 5-8 hours. For convenience, 25ml aliquots of TCBS agar can be stored, melted and poured as required. Isolation media are incubated at 30(C but biochemical testing is undertaken at 37(C. V cholerae can be distinguished from Vibrio alginolyticus, which it resembles on TCBS, by the latter's requirement for NaCl. V alginolyticus is a strict halophile, which will grow in 10% but not 0% NaCl. Suspected isolates of V cholerae should be referred to a reference laboratory for confirmation, serotyping and toxin testing.

Biochemical confirmation

The Enteric Reference Laboratory at ESR confirms isolates using conventional biochemical tests, including indole, MRVP, lysine and ornithine decarboxylase, arginine dihydrolase, and the following carbohydrates: glucose (acid and gas production) L-arabinose, arabitol, cellobiose, lactose, maltose, D-mannitol, salicin and sucrose (Table 1). Salt tolerance is tested using broths with various NaCl concentrations ranging from 0% to 10%. Slide agglutination is performed with O1 and O139 antisera and isolates reactive with anti-O1 are further sub typed using antisera specific for Ogawa, Inaba and Hikojima strains.

All isolates are tested for sensitivity to the vibriostatic agent O129 (2,4 diamino-6,7 diisopropyl pteridine). Sensitivity to this agent was once regarded as a diagnostic feature of Vibrio cholerae serotype O1, and resistance indicative of Vibrio cholerae serotype O139, but isolates of both serotypes may be sensitive or resistant. Resistance is associated with chromosomal insertion of a transposon (3). A 1992 study in India of O129 resistance in 98 consecutively isolated strains of V cholerae O1 biotype El Tor (49 strains each of subtypes Ogawa and Inaba) found that 90% were resistant to 10(g and 82.7% were also resistant to 150(g of O129 (4).

Table 1. Biochemical characterisation of *Vibrio cholerae*

Substrate	% Positive
Indole	99
VP	75
Lysine decarboxylase	99
Arginine dihydrolase	0
Ornithine decarboxylase	99
Glucose acid	100
Glucose gas	0
L-arabinose	0
Arabitol	0
Cellobiose	8
Lactose	7
Maltose	99

D-mannitol	99
Salicin	1
Sucrose	100
Salt tolerance 0%	100
1%	100
6%	53
8%	1
10%	0

Subtyping :

There are over 155 (O) antigenic types of *V cholerae,* but prior to 1992, epidemic cholera was restricted to serotype O1. There are two biotypes of O1, classical and El Tor, which are differentiated by the VP test, sensitivity to Polymyxin B 50U, haemolysis of sheep erythrocytes and agglutination of chicken erythrocytes (Table 2). Bacteriophage typing can also be used to differentiate the biotypes.

Table 2. Differentiation of classical and El Tor biotypes of *V cholerae* O1.

Test	Classical	El Tor
VP	-	+
Polymyxin B 50U	Sensitive	Resistant
Haemolysis sheep rbcs	Non-haemolytic	Haemolytic
Agglutination chicken rbcs	-	+

In addition to serotyping and biotyping, O1 strains can be further characterised by subtyping the minor O antigens or O factors (Table 3). In late 1992, a new serotype causing epidemic cholera emerged in India and Southern Bangladesh (5,6). Serotype O139 is thought to have evolved from a strain of O1 biotype El Tor, by substitution of rfb genes that code for the O1 lipopolysaccharide (LPS) in V cholerae O1 with genes that code for the capsular antigen of V cholerae O139 (7). This serotype, also referred to as the Bengal strain, was responsible for disease in the Indian subcontinent, neighbouring countries in Asia, and imported cases in developed countries. It was the dominant strain of V cholerae for a time but now the El Tor biotype of serotype O1 again predominates.

Table 3. Subtypes of *V cholerae* O1

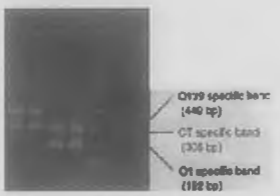
Subtype of 01	Minor antigens/O factors
Ogawa	A and B
Inaba	A and C
Hikojima	A, B and C (this subtype is rare and unstable)

Toxin testing

Cholera toxin (CT) causes the disease of cholera. Toxigenic strains are notifiable in New Zealand and our Ministry of Health is obliged to report them to the World Health Organisation (WHO). CT production is associated with serotypes O1 and O139, both of which cause epidemic cholera. Rarely, CT-negative strains of these serotypes are also found. In addition, CT-production has been reported in serotypes other than O1 and O139, although in considerably smaller amounts than is usual with the epidemic strains (8). Non-O1, non-O139 strains have been associated with sporadic cases of gastroenteritis, including cholera-like diarrhoea, mainly in tropical areas (9).

However, the toxin is encoded by a temperate bacteriophage, CTX((10), which requires the presence of a specific receptor, the toxin co-regulated pilus (TCP). TCP is one of the colonisation factors produced by vibrios, enabling attachment to the gut wall (11). The gene cluster encoding TCP is found on the V cholerae pathogenicity island (VPI). Karolis and colleagues (1999) reported that the VPI is also phage encoded (12), although this has not been confirmed by other workers (13). The possibility remains that any serotype of V cholerae has the potential to acquire the genes for both the receptor and the cholera toxin and therefore strains of V cholerae should be examined for toxigenicity (9). This is done by PCR in the Enteric Reference Laboratory, using primers for the O1 and O139 serogroups of V cholerae, and CT-specific sequences (14), as shown in Figure 1. The toxin itself is very similar to the heat-labile toxin produced by toxigenic strains of E coli and can be demonstrated in tissue culture.

Figure 1. PCR gel showing O1, O139 and CTX- specific bands. New Zealand isolates of *V cholerae*



Toxigenic strains of V cholerae are rarely isolated in New Zealand and infections are invariably acquired overseas. The situation is different for non-toxigenic strains however. Table 4 gives the incidence of both toxigenic and non-toxigenic strains isolated over the last five years.

There have been two Vibrio cholerae isolates in 2004 to date (October). Both were toxigenic strains of O1 biotype El Tor, subtype Ogawa and were resistant to O129 150U. One strain was isolated from a patient in Rotorua and one was isolated from an Auckland patient. Although unconnected, both patients had recently travelled to India.

Conclusions

Strains of Vibrio cholerae capable of causing epidemic cholera are isolated rarely in New Zealand, but a fatal case of cholera did occur in the 1970s. The potential for non-O1, non-O139 strains to cause epidemic cholera exists as the toxin gene is phage encoded and therefore mobile, and the genes encoding the toxin receptor may also be acquired. Recently serotypes other than O1 and O139 have been shown to produce small amounts of cholera toxin. It is important that isolates of Vibrio cholerae are examined for the potential to produce cholera toxin in addition to being serotyped.

Non-O1, non-O139 strains are endemic in New Zealand and may be isolated from faecal samples or infected ears, especially in the summer months. These strains do not produce cholera toxin but they do produce other toxins and may cause diarrhoea. Susceptibility to the vibriostatic agent O129 can no longer be used as an indication of Vibrio species as many strains are now resistant. The serotype V cholerae O1 biotype El Tor, subtype Ogawa is currently the most frequently isolated toxigenic type in New Zealand.

Table 4. *Vibrio cholerae* isolates 1999-2003

Date	Organism	Number	District	Comments
1999	V cholerae non-01, non-O139	4	Waikato: 3 Otago: 1	3 faecal isolates, 1 isolate from otitis media
1999	V cholerae O1 biotype El Tor, subtype Ogawa	1	Central Auckland: 1	Recent overseas travel to Fiji
2000	V cholerae non-01, non-O139	6	Central Auckland: 1 Nth Auckland: 1 Waikato: 4	All faecal isolates
2001	V cholerae non-01, non-O139	5	Waikato: 1 Wellington: 1 Canterbury: 1 Tauranga: 1 Hastings: 1	4 faecal isolates 1 ear isolate
2001	V cholerae O1 biotype El Tor, subtype Ogawa	2	Central Auckland	1 ROT Bali 1 ROT India
2001	V cholerae O1 biotype El Tor, subtype Hikojima	1	Central Auckland	1 ROT China
2002	V cholerae non-01, non-O139	4	Central Auckland 1 Waikato 1 Wellington 1 Blenheim 1	3 faecal isolates 1 ear isolate
2002	V cholerae O1 biotype El Tor, subtype Ogawa	1	Central Auckland	ROT India
2003	V cholerae non-01, non-O139	13	Northland 1 Central Auckland 8 Tauranga 1 Gisborne 1 Manawatu 1 Canterbury 1	11 faecal isolates 2 ear isolates 1 ROT Cambodia 1 ROT not specified
2003	V cholerae O1 biotype El Tor, subtype Inaba	1	Central Auckland	ROT Thailand

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Address for correspondence: Jenny Bennett, Enteric Reference Laboratory, ESR Kenepuru Science Centre, Porirua. Email: Jenny.Bennett@esr.cri.nz

Foetal maternal haemorrhage detection with the Kleihauer technique for postnatal immunoglobulin dose evaluation in Sudan

Mohamed Siddig M Ali, PhD, Assistant Professor and Head of the Department of Haematology; Ahmed Yousif M El Amin, MBBS, Assistant Professor of Haematology; Maysoon Gamal, BS, Teaching Assistant; Nasrin Abdulla, BS, Teaching Assistant; Amal Mohamed, BS, Teaching Assistant.
Haematology Department, Faculty of Medical Laboratory Sciences, El Neelain University, Khartoum, Sudan

Abstract

Objective: The intent of this study was to evaluate the standard routine dose (500 IU) of Rh immune globulin (RHIG) therapy, which is offered routinely to all RhD-negative mothers delivering RhD-positive babies in Sudan.

Methods: Blood samples from 140 pregnant women who were admitted for delivery to various Khartoum State hospitals were tested by the Kleihauer technique to determine the amount of fetomaternal haemorrhage (FMH) in the maternal circulation.

Results: The results of the study demonstrated that the circulation of 10 out of 140 mothers (7.1%), tested by the Kleihauer method, contained more than 4ml of foetal blood. In addition, the association between foetal haemorrhage and mothers' age, duration of pregnancy, baby weight, circumcision of the mother (cutting of the clitoris), type of delivery, and mothers' gravida was statistically insignificant.

Conclusions: The study concluded that 7.1% of mothers had a possibility of greater than 4ml of FMH. For these mothers, the standard RHIG dose of 500IU would be inadequate and they would need additional RHIG to prevent sensitization and potential harm to future babies. It is recommended to test all RhD-negative women delivering RhD-positive babies routinely with the Kleihauer method for detection and quantitation of FMH in order to determine the correct dose of RHIG to be administered.

Key words: Rh immune globulin, Kleihauer technique, foetal maternal haemorrhage, Rh sensitization, Sudan

Introduction

Red cell sensitization is a reversible binding between an antibody and its corresponding red cell antigen. It can occur as a result of the passage of foetal red cells into the maternal circulation transplacentally. Moreover, miscarriage, ectopic pregnancy, blood transfusion and episodes during pregnancy that causes transplacental bleeding, such as amniocentesis and chorionic villus sampling, can also precipitate sensitization (1). Asymptomatic transplacental passage of foetal red cells occurs in 75% of pregnant women either during pregnancy or during labour and delivery. The volume of foetal red cells that enter the maternal circulation also increases as the pregnancy progresses (2). Potential sensitization of mother's red blood cells (RBCs) is determined by the existence of a maternal-foetal blood group incompatibility and by the extent of foetal maternal haemorrhage (FMH). However, the primary immune response, which usually results in the production of IgM antibodies, is often weak. IgM antibodies do not cross the placenta. Therefore, ABO haemolytic episodes, most often caused by IgM antibodies, are usually mild and rarely responsible for foetal death (2-4).

However, RhD antibodies are primarily IgG and are able to cross the placenta once the primary response has developed. Subsequent exposure to RhD-positive RBCs produces a rapid increase in anti-D antibodies, which are primarily IgG, resulting in haemolytic disease of the new born (2,4). The disease occurs when RhD-negative mothers, immunized to RhD-positive RBCs during a previous pregnancy become pregnant again with an RhD-positive foetus (5). Haemolytic disease of the new born due to RhD incompatibility has become much less common following the introduction of anti-D prophylaxis (1), the dose for which depends upon the size of the foetal maternal haemorrhage as determined by the Kleihauer technique (6). Since primary immunization during pregnancy can precipitate sensitization, underestimation of the volume of transplacental haemorrhage is the major cause of treatment failure due to inadequate dose of Rh immune globulin (RHIG) (5).

Severity of the disease depends upon the nature of the individual's immune response, which may range from mild haemolysis to severe anaemia with compensatory hyperplasia of erythropoietic tissue leading to massive enlargement of the liver and spleen (4). The standard dose of 500 IU of RHIG given in Sudan to RhD-negative mothers antenatally is lower than that used in Europe, the United States and Canada, where 1000-1500 IU is the standard dose (7).

This dose is given to all RhD-negative mothers without measuring the volume of transplacental haemorrhage. However, adjustment of this dose according to the amount of foetal maternal haemorrhage of mothers is mandatory. Since this treatment is expensive, many of these mothers may not have the money for this treatment and accept an inadequate dose, which may result in sensitization with its unfavourable consequences.

The Kleihauer technique, based on acid elution of maternal red cells, is the most widely used technique for screening and estimating the volume of FMH and for determining the need for additional doses of RHIG to prevent maternal allo-immunization (8). In Sudan, this test is mainly used for the diagnosis of haemoglobinopathies. The test is simple and inexpensive and can be routinely performed by any hospital laboratory in Sudan.

The present study was intended to evaluate the effectiveness of the standard dose, which is given to all RhD-negative mothers with RhD-positive babies since, according to our knowledge, such studies have not been previously conducted in Sudan.

Materials and methods

The design of the study is descriptive, cross-sectional and facility based. It was conducted in the El Neelain University laboratories using a total of 140 maternal blood samples collected from the Bahri Hospital, the Maternity Hospital, and the Ibrahim Malik Hospital. Samples were collected between March and September 2004. All RhD-positive mothers (n=30) and RhD-positive mothers (n=110) who were admitted for delivery to these hospitals during the study period and who delivered an RhD-positive baby were included in the study. Two ml of blood were collected from each subject (RhD-negative mothers who

delivered RhD-positive babies) in EDTA blood collection tubes.

Thin blood films were prepared from each blood sample, and then stained using an acid-elution cytochemical method, which was introduced by Kleihauer and colleagues (6), and examined for foetal red cells. The technique was modified by increasing the reaction time and incorporation of new methylene blue in the buffer solution used for washing the films. A positive control (cord blood) and negative control (adult male blood) were processed in parallel with the mother's blood samples.

Elution solution A was prepared by adding 7.5g/l haematoxylin to 90 % ethanol while solution B was prepared by dissolving 24 g of FeCl3 in 20 ml of 2.5 mol/l HCl and bringing to one litre with distilled water. Five volumes of solution A were mixed to one volume of solution B for the working elution solution. The counter stain was prepared by dissolving 2.5 g of the aqueous eosin in one litre of distilled water.

The fresh air-dried thin blood films were fixed for 5 minutes in 80 % ethanol in a Coplin jar. Next, the slides were rinsed rapidly in distilled water and left vertically on blotting paper for about 10 minutes to dryness. The slides were then placed for 20 seconds in a Coplin jar containing the working elution solution and then rinsed in tap water before being allowed to air-dry. Finally, the slides were stained in a Coplin jar containing eosin for two minutes and washed with tap water.

The stained slides were then examined microscopically using a high dry objective lens (40X) and foetal cells were counted against ghost cells. The foetal haemorrhage was calculated as follows:

Uncorrected volume of bleed: 1800ml x foetal cells counted (F) / adult cells counted (A). Where 1800 is the presumed maternal cell volume.

Correction for foetal volume: (J) = (1800ml x F/A) x 1.22 (foetal cells are 22% larger than maternal cells)

Correction for staining efficiency: FMH = J x 1.09 (only 92% of the foetal cells can be detected).

Results

The Kleihauer technique was performed on a total of 140 maternal blood samples collected within 72 hours after delivery. Blood samples were categorized into two groups. The major group included the Rh-D positive mothers, which accounted for 110 of the total number. The second group included 30 RhD-negative mothers. The ages of 135 mothers were between 18 and 45, while 5 mothers were less than 17 years old. Thirty of the mothers were not circumcised and were RhD-negative, while 110 were circumcised and were RhD-positive. Forty of the mothers were primigravida and 60 were delivered by caesarean section versus 100 multigravida and 80 mothers delivered normally. Ten mothers had a foetal maternal bleed of greater than 4ml while the other 130 had foetal maternal bleeds of less than 4ml.

Table 1. Correlation between mother's age and foetal maternal haemorrhage

Mother's age		Foetal haemorrhage		Total	Average
		0 - 4 ml	> 4 ml		
0 - 17 Years	Number	4	1	5	
	Mean	0.13	14.2		7.1
18 - 45 Years	Number	126	9	135	
	Mean	0.54	14.98		7.76
Total	Number	130	10	140	
	Mean	0.34	14.59		7.47

Four mothers less than 17 years old had a foetal maternal haemorrhage of less than 4ml, while only one mother in this group had more than a 4ml foetal maternal haemorrhage. Nine mothers in the 18-45 year old group (6.7%) had a foetal maternal haemorrhage of more than 4ml,versus 126 (93.3%) with less than a 4ml bleed. However, the correlation between mother's age and amount of foetal maternal haemorrhage was not statistically significant (P=0.256).

Table 2. Correlation between type of delivery and foetal maternal haemorrhage

Type of delivery		Foetal haemorrhage		Total	Average
		0 - 4 ml	> 4 ml		
Normal	Number	76	6	82	
	Mean	0.58 1	6.65		8.62
Caesarean	Number	54	4	58	
	Mean	0.45	12.28		6.37
Total	Number	130	10	140	
	Mean	0.52	14.47		7.50

Six mothers (7.5%) who delivered normally were found to have more than 4ml of the foetal maternal haemorrhage. Four mothers (6.7%) delivered by caesarean section. However, the association between the type of delivery and foetal maternal haemorrhage was not statistically significant (P=0.85)

Table 3. Correlation between mother's Rh blood group and foetal maternal haemorrhage

Mother's Rh blood group		Foetal haemorrhage		Total	Average
		0 - 4 ml	> 4 ml		
Negative	Number	30	0	30	
	Mean	0.73	0		0.37
Positive	Number	100	10	110	
	Mean	0.51	14.9		7.71
Total	Number	130	10	140	
	Mean	0.62	7.45		4.04

The association between the mother's Rh blood group and foetal maternal haemorrhage failed to reach statistical significance (P=0.087). All mothers who were RhD-negative had less than 4ml of foetal blood, while 10 (10%) of the RhD-positive mothers had more than 4ml of foetal blood.

Table 4. Correlation between circumcision of mothers and foetal maternal haemorrhage

Circumcision of mothers		Foetal haemorrhage		Total	Average
		0 - 4 ml	> 4 ml		
Circumcised	Number	28	2	30	
	Mean	0.48	17.2		3.20

Non-circumcised	Number	102	8	110	
	Mean	0.69	5.7		8.84
Total	Number	130	10	140	
	Mean	0.59	11.45		6.02

Only two of the circumcised mothers (6.7%) were found to have more than 4ml of foetal maternal haemorrhage post delivery, versus eight non-circumcised mothers (7.3%). However, no statistically significant association was found between circumcision of mothers and foetal maternal haemorrhage (P=0.909).

Table 5. Correlation between mother's gravida and foetal maternal haemorrhage

Mother's gravida		Foetal haemorrhage		Total	Average
		0 - 4 ml	> 4 ml		
Primigravida	Number	37	3	40	
	Mean	0.42	25.5		12.96
Multigravida	Number	93	7	100	
	Mean	0.56	10.36		5.46
Total	Number	130	10	140	
	Mean	0.49	17.93		9.21

Three of 40 primigravida (7.5%) mothers were found to have more than 4ml of foetal maternal haemorrhage, compared to seven of 100 (7%) of multigravida mothers. No statistically significant correlation was found between the mother's gravida and the amount of foetal maternal haemorrhage (P=0.917).

Table 6. Correlation between baby's weight and foetal maternal haemorrhage

Baby's weight		Foetal haemorrhage		Total	Average
		0 - 4 ml	> 4 ml		
2-3 Kg	Number	77	6	83	
	Mean	0.48	16.65		8.57
3-4 Kg	Number	50	4	54	
	Mean	29.7	12.28		20.99
4-5 Kg	Number	3	0	3	
	Mean	0.47	0		0.24
Total	Number	130 1	0	140	
	Mean	10.22	9.64		9.93

Out of 83 mothers who delivered babies weighing 2-3 Kg, six of them (7.2%) were found to have more than 4ml of the foetal haemorrhage as well as four mothers (7.4%) who delivered babies weighing 3-4 Kg. No foetal haemorrhage was detected in mothers who delivered 4-5 Kg weight babies. No statistically significant correlation was found between baby's weight and the amount of foetal maternal haemorrhage (P=0.655)

Table 7. Correlation between duration of pregnancy and foetal maternal haemorrhage

Duration of pregnancy	Foetal haemorrhage			Total	Average
		0 - 4 ml	> 4 ml		
7 months	Number	1	0	1	
	Mean	1	0		0.5
8 months	Number	2	1	3	
	Mean	0	5.2		2.6
9 months or more	Number	127	9	136	
	Mean	0.52	15.98		8.25
Total	Number	130	10	140	
	Mean	0.51	7.06		3.78

One mother of the three who carried their babies for 8 months was found to have more than 4ml foetal haemorrhage as well as nine mothers (6.6%) carrying their babies for 9 months. All mothers who carried their babies for 7 months had less than 4ml of foetal maternal haemorrhage. However, no significant correlation was detected between the duration of pregnancy and the amount of foetal maternal haemorrhage (P=0.198).

Discussion

Sensitization of women of childbearing age has serious consequences on subsequent pregnancies. Therefore, the administration of a suitable dose of RHIG is recommended within 72 hr after delivery for the prevention of sensitization to the RhD antigen and development of RhD antibodies. In Sudan, a constant dose of 500 IU is given routinely to all RhD-negative mothers who deliver RhD-positive babies regardless of the severity of sensitization, which is closely correlated with the number of foetal cells that enter the mother's circulation. The number of foetal cells in the maternal circulation is used for the determination of the foetal maternal haemorrhage, which in turn determines the correct dose of RhD antibodies. The routine dose of 500 IU of RHIG can only be justified when foetal maternal haemorrhage is less than 4ml. However, this dose is unsatisfactory when mothers have a foetal maternal haemorrhage of more than 4ml. As a result, mothers become sensitized and produce antibodies that cross the placenta to the foetal circulation during subsequent pregnancies and cause spontaneous abortion or hydrops fetalis. This not only results in an increase in morbidity and mortality, but also an increase in costs to the healthcare system.

Although most of the mothers who participated in this study were found to have less than a 4ml foetal maternal haemorrhage (93%), the smaller portion (7%) that was found to have more than 4ml haemorrhage is considerable. This implies that the routine dose of RHIG is unsatisfactory for 7% of Sudanese mothers. Therefore, they are at a higher risk for complications. Thus, the performance of Kleihauer test within 72 hours after delivery will undoubtedly help to determine the appropriate RHIG dose to be offered to these mothers.

Moreover, the application of this technique is easy, rapid and has a low cost. It can be utilized in all hospitals in the Sudan, including regional and outlying hospitals. This conclusion is in agreement with the comments of Ghosh and Murphy (9) who determined that failure to give adequate postnatal RHIG is the most identifiable cause of

sensitization in four of 70 cases in Scotland between 1985 and 1990.

In the present study, the correlation between the foetal maternal haemorrhage and mother's age, type of delivery and baby weight was found to be insignificant, which illustrates that mothers with variable age ranges are equally susceptible to the passage of foetal cells into the maternal circulation. Furthermore, the number of foetal cells entering the mother's circulation does not depend on the type of delivery or the weight of the baby.

Although circumcision of mothers is known to increase the difficulty of delivery, its association with foetal maternal haemorrhage was not significant, circumcision does not contribute to the number of foetal cells in the maternal circulation.

This study also showed that the mother's RhD blood group is not associated with the amount of foetal maternal haemorrhage. This is seen in the observed insignificant statistical difference between the mother's RhD blood group and the number of detected foetal cells. Furthermore, duration of pregnancy was not significant for foetal maternal haemorrhage. Similarly, foetal maternal haemorrhage was not related to the mother's gravida; multi- or primigravida mothers had equal foetal maternal haemorrhages.

In conclusion, the number of RhD-negative mothers in Sudan with more than 4ml foetal maternal haemorrhage (7%) is considerable. Determination of foetal maternal haemorrhage after delivery is mandatory in order to determine the appropriate dose of RHIG. Foetal red cells entering the mother's circulation was not associated with the mother's age, duration of pregnancy, circumcision, baby's weight, mother's gravida, RhD blood group, nor type of delivery.

It is recommended to perform a Kleihauer technique as a routine test to determine the amount of foetal cells in the mother's circulation within 72 hours after delivery on all RhD-negative mothers delivering RhD-positive babies. The RHIG dose should be based on the amount of foetal maternal haemorrhage.

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Correspondence: Mohamed Siddig Mohamed Ali. Faculty of Medical Laboratory Sciences, Al Neelain University, P.O. Box 12702, Khartoum, Sudan. E-mail: mohdaru@hotmail.com

Haematology

Special Interest Group

HSIG questionnaire

Journal - Blood Reviews - Volume 17, Number 4, December 2003, Pages 209-213 Congenital Neutropenia

Questions

Q.1 Define congenital neutropenia

Q.2 Name 2 underlying clinical features seen in congenital neutropenic children

Q.3 State 3 causes of leukaemic transformation

Q.4 What is the most common characteristic of cyclical neutropenia?

Q.5 G-CSF treatment increases the amplitude of monocyte, lymphocyte and neutrophil oscillation, shortens both the cycle length and duration of monocytopenia - True/False

Q.6 Shwachman-Diamond Syndrome (SDS) is a sex linked disease characterized by skeletal abnormality - True/False

Q.7 Name 4 clinical features associated with SDS

Q.8 Glycogen storage disease type results from deficiency of the ..... enzyme (GSD16)

Q.9 GSD16 transports glucose-6-phosphate into the endoplasmic reticulum for conversion to glucose and phosphate - True/False

Q.10 State 4 rare causes that contribute to congenital neutropenia

Q.11 Myelokathexis is an autosomal dominant moderate to severe neutropenia with an unusual morphology - True/False

Q.12 .....is characterized by unfortunate combination of neutropenia and cardiomyopathy

Q.13 Pearson's syndrome manifests as .....with vacuolization of bone marrow precursors, ...and variable degrees of neutropenia and thrombocytopenia

Answers on page...20

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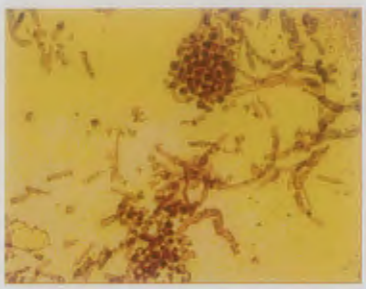

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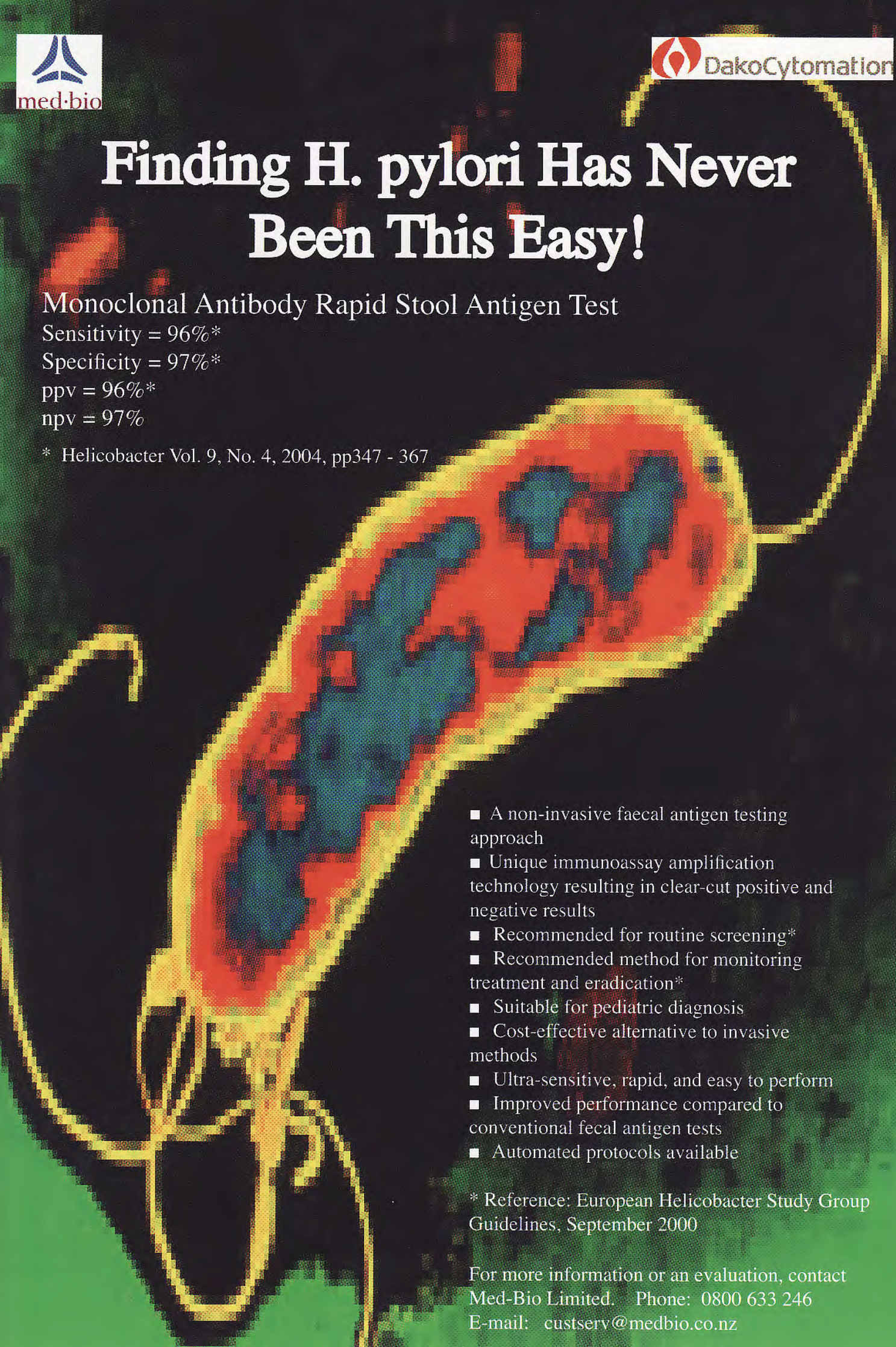

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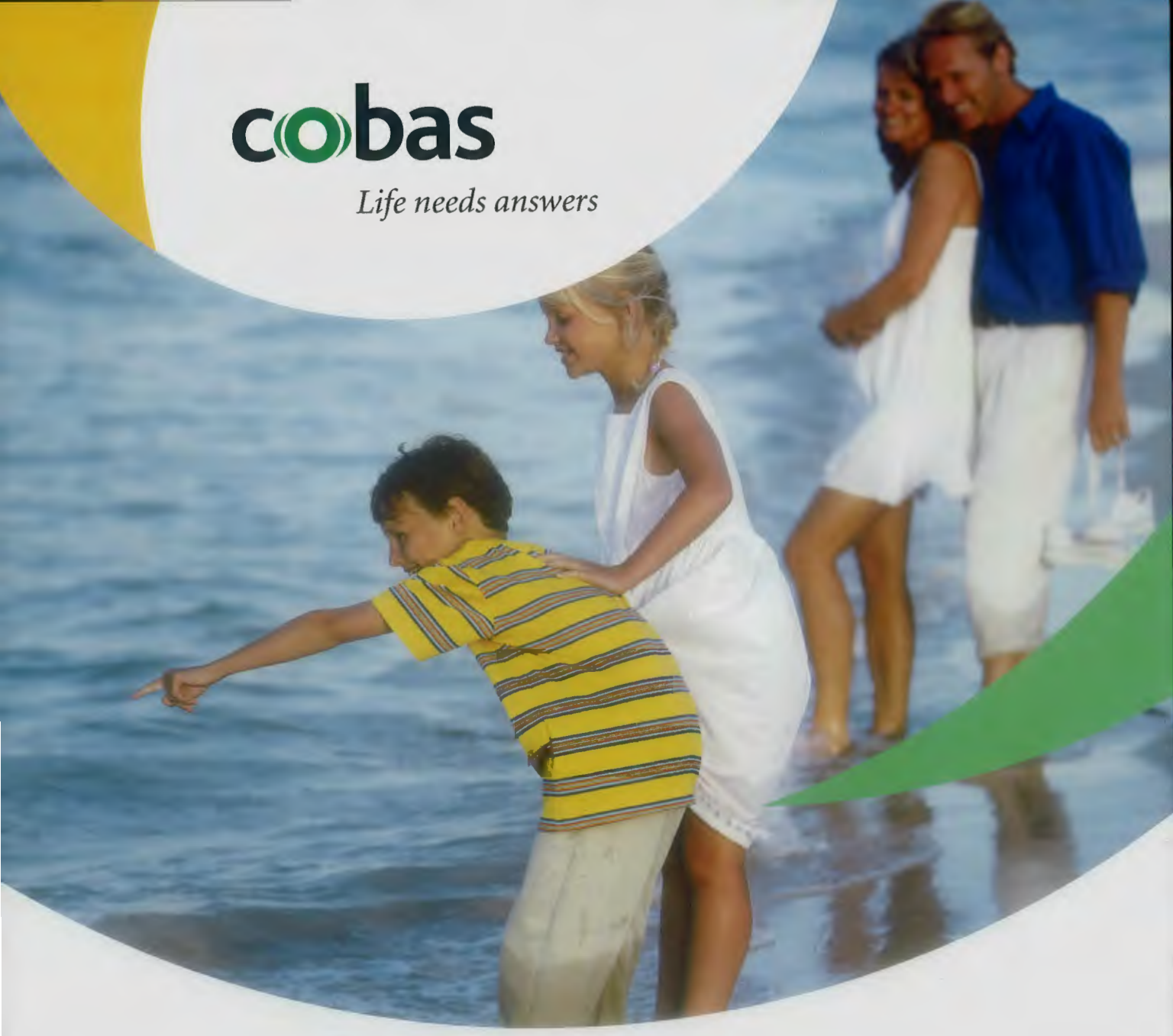

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