NATIONAL BLOOD TRANSFUSION SERVICE

South Western Regional Transfusion Centre
Southmead Road
Bristol BS10 5ND
Telephone:
0272-507777 Telex: 449384

Dr. I. D. Fraser, Consultant Haematologist & Director
Dr. T. B. Wallington, Consultant Immunologist
Dr. D. J. Anstee, Top Grade Scientific Officer
Mr. Peter Brooman, Administrator

TBW/ms

17th April 1984.

Mr. R.D. Smart
Chairman
CENTRAL BLOOD LABORATORIES AUTHORITY
The Crest
Blood Products Laboratory
Elstree
Boreham Wood WD6 3AU

Dear Mr. Smart,

As a consequence both of discussions within the Research Committee formed by your Authority and one of its off shoots, the AIDS Working Party, I have put together a detailed application for support from the Medical Research Council for a Research Project aimed at evaluating screening tests for antibody to Hepatitis B core antigen as a screen to exclude blood donors who present a high risk of transmitting AIDS. The project, if funded, will be based on the South Western Regional Transfusion Centre and the North London Transfusion Centre at Edgware. It will involve medical follow up of blood donors who prove positive in the laboratory in order to establish the risk that they present. Other tests of a kind that might be routinely performed in a Transfusion Centre will also be used to see whether they help in discrimination.

I enclose a copy of the application for your information. It is not complete in every detail as the participation of the North London Blood Transfusion Centre is not properly acknowledged as I am still involved in discussions with them. However the major intent of the project and all the detailed and costed propositions are there and the M.R.C. was happy to consider it at this stage.

If you have any queries or comments please do not hesitate to contact me.

Yours sincerely,

GRO-C

T.B. Wallington
Consultant Immunologist

79/5

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MRC
Medical Research Council

Please type throughout ar
attach seven photocopi

Applicatio for a project grar

1. Applicant(s) Surname(s) | Forename(s) | Age(s)

	Surname(s)	Forename(s)	Age(s)
(a)	WALLINGTON	TIMOTHY BADEN	GRO-C
(b)			
(c)			

Post held (a) (b) (c)

2. (a) Institution/Authority (administering grant if approved)

City or Town

(b) Department accommodating project (and institution if other than opposite)

3. Title of project

An evaluation of screening tests for anti HBc in the detection of blood donors at risk of transmitting AIDS.

4. Type of grant sought
New Project Grant
(Health Services Research)

5. No. of hours per week applicant(s) will devote personally to this project: (a) 4 (b) (c)

6. Abstract of research

Serological evidence of infection with Hepatitis B is found in the majority of AIDS patients and persons in groups at high risk of contracting AIDS. These persons are a danger as blood donors as there is clear evidence of the transmission of AIDS by the transfusion of blood and blood products. This project plans to evaluate screeni for anti HBc, a test familiar in the Blood Transfusion Laboratory, in identifying at risk persons by personal interview of positive donors and suitable controls. Other non specific abnormalities reflecting disorder in the immune system are found in AIDS. Some might prove appropriate as screening tests. Immunoglobulin levels, circulating immune complexes, interferon alpha, Beta-2 microglobulin, TPHA and serology for HTLV will be studied in anti-HBc positives and controls looking for further information which discriminates at risk persons.

For Office use											

7. Proposed starting date: 1.11.84.

Proposed duration (in months): 24

8. Summary of support requested

		First year £	Second year £	Third year £	Total over peri £
(a) Personal support of applicant		nil	nil	nil	nil
(b) Research staff (medical/scientific)	Number of posts: Whole/part-time 1 whole 1 part time	13405	13881	nil	27286
(c) Technical/other assistance	Number of posts: Whole/part-time 3 whole 1 part time	16145	16304	nil	32449
Addition for superannuation and National Insurance calculated as 25% of salaries in (a), (b) and (c)		7388	7546	nil	14934
(d) Visiting senior scientist (salary/subsistence, airfare)		nil	nil	nil	nil
(e), (f), (g) Expenses		6970	6970	nil	nil
Total recurrent		43908	44701	nil	88609
(h) Apparatus		1902	nil	nil	1902
Total support requested		45810	44701	nil	90511

4/9/6

9. (a) Is your related research currently being supported by any outside body (other than the MRC)? If so, which organisation and what support is provided (including its tenure)

NO

(b) Are you currently applying elsewhere for support for work relating to the present proposal? If so, to which organisation?

NO

(c) Is this application currently being submitted elsewhere? If so, to which organisation; and by what date is a decision expected?

NO

(d) Has this application been submitted elsewhere over the past year? If so, to which organisation and what was the result?

NO

(e) Is the proposed research likely to lead to patentable or otherwise commercially exploitable results? If so, has it already been discussed with NRDC or any commercial organisation?

NO

10. Full official postal address of applicant

SOUTH WESTERN REGIONAL TRANSFUSION CENTRE
Southmead Road
Bristol BS10 5ND

Telephone number of applicant (please give STD code from London and extension)

GRO-C

11. Acceptance of regulations and conditions

I have read the conditions set out in the Council's current Projec Grants pamphlet and, if my application is successful, I agree to abide by them. I shall be actively engaged in, and in day-to-day control of, the project.

Signature of applicant(s): GRO-C

Date: 12·4·84.

12. This application should be submitted by/through (i) the Head of Department and (ii) the officer who w: be responsible for administering any grant that may be awarded. Each should sign the followin declaration:

I confirm that I have read this application and that, if granted, the work will be accommodated an administered in the Department/Institution in accordance with the conditions in the Council' current Project Grants pamphlet. The **staff gradings and salaries** quoted are correct and ir accordance with the normal practice of this Institution.

(i) Signature of **Head of Department**

GRO-C

Title CONSULTANT IMMUNOLOGIST

To be appended in typescript or block capitals

Name and initials (of (i) above)

T.B. WALLINGTON

Institution

S.W. REGIONAL TRANSFUSION SERVICE

Address (if different from 10 above)

Date: 12th April 1984

(ii) Signature of **Administrative Authority**

GRO-C

Finance Officer/Bursar/Hospital Secretary

To be appended in typescript or block capitals

Name and initials (of (ii) above)

J.H. DUFTY

Institution

S.W. REGIONAL TRANSFUSION SERVICE

Address and telephone number (including STD code from London and extension)

Southmead Road
Bristol BS10 5ND
Tel: GRO-C

Date: 12th April 1984

13. Name, address and telephone number (including STD code from London and extension) in typescript (or block capitals) of the officer who should be contacted regarding the administration of the grant if awarded, if different from (ii) above:

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PROPOSED INVESTIGATION

1. Title of project
2. Purpose of proposed investigation
3. Background of the project
4. Plan of investigation
5. Detailed justification for support requested

These headings must be adhered to in the case of new project grant applications.
In the case of applications for:
(i) supplementation/extension of an existing grant
(ii) visits to learn special techniques
(iii) collaborative visits abroad
(iv) visiting senior scientists
(v) shared equipment
please refer to the yellow working copy.

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TITLE

An evaluation of screening tests for anti HBc in the detection of blood donors at risk of transmitting AIDS.

PURPOSE OF PROPOSED INVESTIGATION

It is clear that the Acquired Immunodeficiency Syndrome (AIDS) can be transmitted by blood and blood products (1, 2). The causative agent of AIDS is unknown and there are no measurable specific pathological features. However a number of non-specific abnormalities are found commonly in AIDS cases and healthy people members of groups where the risk of developing AIDS is high (3). Antibodies to Hepatitis B Core antigen (Anti HBc) are the commonest abnormality. They are also detectable in a small proportion (0.5 - 2%) of blood donors in the U.K. (4 - 5). The purpose of this project is to evaluate whether this identifies blood donors belonging to groups where the risk of developing AIDS is high and therefore the risk of transmitting AIDS is high. Anti-HBc positive donors and matched controls will be interviewed to discover whether their personal behaviour makes them a member of an high risk group. Tests will be performed for certain of the other abnormalities associated with AIDS to establish their relationship to the risk status of the donor and their value, if any, when combined with a positive anti-HBc test as an extra discriminant of the donor especially at risk of transmitting AIDS.

BACKGROUND OF THE PROJECT

The transmission of infection from donor to recipient is one of the major risks of blood transfusion. Although the cause of AIDS is unknown its epidemiology suggests strongly that an infectious agent is responsible (6). Transmission is by blood, blood products, blood contaminated instruments and sexual intercourse particularly anal intercourse, a situation very reminiscent of Hepatitis B a serious problem in blood transfusion before screening tests were introduced. These routes of infection largely confine AIDS to persons whose life style puts them at high risk, (high risk groups, homosexual/bisexuals, intravenous drug abusers, haemophilliacs) these include recipients of blood and products if persons with AIDS are accepted as blood donors.

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Transmission of AIDS by the transfusion of blood and blood products has been clearly demonstrated (1, 2) proving the reality of this risk.

Symptomatic AIDS is a well established syndrome; Kaposi's sarcoma, opportunistic infection or both in a patient with profound idiopathic acquired deficiency of cellular immunity. These persons are ill and unlikely to be accepted as blood donors. Their clinical problems stem from immunodeficiency which must at first be asymptomatic before the malignancy or infection that it allows develops. Evidence gathered from several sources, particularly imprisoned intravenous drug abusers (7) and transfusion associated cases (1) indicate that the latent period from the time of presumed infection to established immunodeficiency is between 4 months and 4 years. Discrete episodes of homosexual activity in high risk areas imply a similar figure for sexually acquired disease. Are these persons with latent AIDS infectious? The details of donors where blood transfusion transmitted AIDS suggest strongly that they can be (1). These persons should not be accepted as blood donors. The Blood Transfusion Service attempts to exclude them by asking people whose life style puts them at high risk of catching AIDS not to give blood. Risks would be greatly decreased if these donors could be positively identified by screening tests particularly if latent infection could be identified. Naturally there is great concern within the National Blood Transfusion Service that this should be achieved. The Central Blood Laboratories Authority has set up a Working Party to approach this and other problems associated with AIDS. The proposals for this project are the result of discussion within this Working Party.

How might screening be approached? The analogy between the putative AIDS agent and Hepatitis B is more than illustrative. Persons within high risk groups show evidence of infection with this virus and other agents with similar modes of infectivity. Screening tests for these infections might be used to detect them. A number of other abnormalities indicators of disorder within the immune system have also been described in these persons which might prove useful as screening tests. None of these tests are specific for AIDS and their value will only be established by trial in blood donors. To be useful they must meet certain criteria:- they must identify at risk donors without excessive loss of donations due to positives in donors not at risk, technically they must fit into transfusion practice not leading to the loss of donations through unacceptable delays.

Screening for Hepatitis B is well established. In addition to tests for HBs Ag well tried tests for antibody to core antigen (Anti HBc) are available and indicate past as well as present infection.

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Pilot studies of screening for anti HBc in two Regional Transfusion Centres (4, 5) show that it is possible to screen large numbers of blood donations within the limitations imposed by Transfusion practice. A small number of donors (0.5 - 2%) have positive results. Results reported from the Centres for Disease Control, Atlanta (3) show a very high prevalence of this antibody in AIDS patients (84.2%), patients with probable AIDS (78.1%) and asymptomatic homosexuals/bisexuals (80.3%). Studies in London record a lower but substantial incidence (45%) of homosexual men having anti HBc and or anti HBs (8). AIDS patients show marked disorder of B lymphocyte function with increased spontaneous immunoglobulin production (9). This may be why they commonly display high titres of antibody to past infections a finding helpful to screening. For these reasons we have chosen to evaluate screening for anti HBc in the detection of blood donors at risk of transmitting AIDS. The significance of positive results will be investigated by interviewing these donors and matched controls in order to establish whether or not they belong to at risk groups. Further tests will be performed on these samples with the object of increasing discrimination for at risk donors through clustering of positives.

These other tests have been chosen on the following basis:

serum immunoglobulins; IgG, IgA, IgM. These are commonly raised in AIDS where B lymphocyte dysfunction is well described. They are easily measured in the serum sample routinely available from blood donors.

circulating immune complexes Raised concentrations of circulating immunoglobulins as detected by Staph A and C1q based tests are common in AIDS patients (65%) and at risk homosexuals/bisexuals (83%) (3). They can be measured in the serum sample routinely available from blood donors.

B-2- microglobulin This serum protein, possibly a product of leucocyte turnover, is raised in AIDS patients (69.3%) and at risk homosexuals/bisexuals (21%) (3). It can be measured in the serum sample routinely available from blood donors.

Interferon alpha Increased titres of interferon alpha as measured by biological assay have been reported in AIDS patients (10). Recently an immunoassay based on a monoclonal antibody to interferon has been described (11). This can be applied to the serum sample routinely available from blood donors.

Treponema pallidum haemagglutination (TPHA) This test is used routinely for screening in some Transfusion Centres. It has value to this study as further evidence of past exposure to venereally transmitted infection one of the risks associated with AIDS.

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Antibody to Human T Cell leukaemia virus (HTLV) HTLV is an agent especially attractive as the cause of AIDS as it is a retrovirus showing tropism for T helper phenotype cells, the population of immunocytes depleted in established AIDS. 25% of a series of male homosexual AIDS patients had antibody to HTLV (13) whilst it was present in only 1 of 81 matched controls. This does not suggest high utility as a screen for high risk status. This test is included in our protocol as providing information of potential value to epidemiological studies in other risk areas.

Lymphocyte numbers will be measured when positive donors and controls are followed up. Suitable samples are not available at routine donations. 33% of symptom free homosexuals were found to be lymphopenic in a recent series in London (8).

Plan of Investigation

Samples are routinely taken as part of blood donation for blood grouping and other serological testing and also for hepatitis and syphilis screening. Using these samples 50,000 donations will be screened for anti HBc. Testing will be at two Regional Transfusion Centres (North London Transfusion Centre, Deansbrook Road, Edgware and South Western Regional Transfusion Centre, Southmead Road, Bristol). Complete blood donor clinics will be screened both new and old donors. Clinics will be selected so that people from every social and ethnic background are included in the study. The Regional Transfusion Centres concerned have been selected as they employ staff familiar with anti HBc testing and display an incidence of anti HBc 0.5% Bristol, 2% Edgware (4,5) which reflects their differing donor populations and is likely to represent the totality of donors in the U.K. Testing for anti HBc will be by a one step solid phase radio-immunoassay using reagents and controls supplied by Dr. Philip Mortimer, Central Public Health Laboratory, Colindale. Positive specimens will be referred to Dr. R. Tedder, Middlesex Hospital for check testing.

This screening is likely to yield around 500 anti-HBc positive donors (4-5). Positive donors will be matched by controls of similar age and sex obtained from the same donor clinic. The routine serum sample used for screening will be aliquoted into 200 ul vials and stored in a vapour phase of liquid nitrogen to await further testing.

Both positive donors and controls will be traced and interviewed by medical staff. Records made will not be identifiable by name. It will be necessary to delve into personal matters in order to establish the 'at risk status' of each individual. Complete confidentiality will be assured. At this time a further blood sample will be taken for repeat tests and full differential white blood cell count.

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Further laboratory tests on aliquoted specimens will be performed as follows:

Immunopathology Laboratory, South Western Regional Transfusion Centre.

Immunoglobulins using the standard method of radial immunodiffusion in plates prepared in the laboratory.

Immune complexes three assays part of the routine practice of the laboratory will be used.

Fluid phase radiolabelled Clq binding. The standard assay modified to EDTA rather than heat pretreatment of samples.

Platelet aggregation test. A test for IgG containing immune complexes developed by Penttinnen(12).

Poly.ethylene.glycol (PEG) precipitation. In this simple test proteins are precipitated from serum by PEG 6000 at a final concentration of 2%. The precipitate is washed and then redissolved in buffer. The total protein in the precipitate is measured in a spectrophotometer, high levels are taken as indicating the presence of immune complexes.

Interferon alpha An immuno radiometric assay based on monoclonal antibody to interferon and supplied as a kit by Boots Celltech Diagnostics will be used.

B-2-microglobulin An enzyme linked immunoassay supplied as a kit will be used. To be purchased from Phremacia Diagnostics.

Department of Microbiology, North London Transfusion Centre.

Treponema pallidum haemagglutination Samples will be screened using the method routine for all blood donor samples in this Transfusion Centre.

Middlesex Hospital Dr. R. Tedder Samples will be sent to the Department of Virology for check anti HBc estimations and antibodies to HTLV by routine methods.

All of these 'further tests' will be repeated using the follow up blood sample.

Pilot studies carried out at the South Western Regional Transfusion Centre show that it is possible to obtain sufficient aliquots for all of these tests from the serum sample collected routinely from each blood donor for microbiological screening.

Plasma from anti HBc positive donations and controls will be retained frozen in view of its potential value in future research. The plasma reduced red cells will be used routinely unless a standard contraindication exists.

Detailed justification for support requested

Research Staff

1) Medical Registrar This will be a key appointment charged with the day to day management of the project, donor follow up in the Transfusion Centre on which the post is based and collation of results. The post will allow for training in blood transfusion medicine or community medicine according to the career intentions of the doctor appointed. Suitable training contacts and honorary status will be arranged.

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2) Medical Assistant (part time) will be needed in the other Transfusion Centre to follow up donors. This study is based on two Transfusion Centres to assure that all groups within the British population are sampled.

TECHNICAL AND OTHER ASSISTANCE

1) M.L.S.O. staff Considerable use will be made of staff currently in post in the Microbiology Departments of both Transfusion Centres and the Immunopathology Department in Bristol, however Junior M.L.S.O. support is required for anti HBc screening in both Centres. Aliquoting, freezing and later appropriate distribution of specimens in both Centres. Also support with the many additional tests to be undertaken in Immunopathology in Bristol.

2) Secretary (half time) Required to facilitate the most vital component of this project, tracing and contact with donors both anti HBc positive and controls. Appointments for visits, record of visits, correspondence with General Practitioners and Hospital Consultants and general record keeping will be necessary and be the Secretary's task. More than 1,000 donors will be interviewed in detail during the two year period.

Materials and Consumables.

Anti HBc test Reagents for this test will be supplied by the Central Public Health Laboratory Service, laboratory disposables including Dynatech "Removawell" microlitre plates will be purchased. The test can be performed for slightly less than 10p per assay on this basis, this including the necessa controls. 50,000 initial screening assays are anticipated plus 1,000 repeat tests at follow up.

Tests for circulating immune complexes, immunoglobulins. Costing is for the purchase of reagents to perform 2,000 tests in each category.

Interferon alpha Costing is for the purchase of kits to perform 2,000 tests. A discount based on the size of the order is included in the price quoted by the manufacturer.

Beta-2 microglobulin Costing is for the purchase of kits to perform 2,000 tests.

Travel expenses It is anticipated that many donors will not be able to travel to the Regional Transfusion Centre for follow up interview having given blood at a mobile clinic in their immediate locality. Medical staff will have to travel to them for this purpose.

Apparatus A small liquid nitrogen refrigerator plus inventory system will be required in each of the two Transfusion Centres involved in order to store aliquoted specimens awaiting transfer to other laboratories for further testing. Specimens will be snap frozen and aliquoted as soon as possible after initial screening. They will only be thawed immediately before use. This is necessary for immune complex tests. Recent results using the interferon alpha assay (11) also suggest that this is necessary for this substance.

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REFERENCES

1) Curran J.W. et al. (1984) 'Acquired immunodeficiency syndrome (AIDS) associated with transfusions. N. Engl. J. Med. 310 p. 69.

2) Daly H. Scott G.L. (1983) 'Fatal AIDS in a haemophiliac in the U.K.'. Lancet ii p. 1190.

3) Data presented by C.D.C. Staff at the WHO AIDS Meeting, Geneva, Nov. 1983.

4) Archer A.C. et. al. (1983) 'The value of screening blood donors for antibody to hepatitis B core antigen'. J. Clin. Path 36 p. 924.

5) Tedder R.S. et. al. (1980) 'Contrasting patterns and frequency of antibodies to surface, core and e antigens of hepatitis B virus in blood donors and in homosexual patients'. J. Med. Virol 6 p. 323.

6) Pinching A.J. (1984) 'The acquired immune deficiency syndrome'. Clin. exp Immunol 56 p. 1.

7) Wormser G.P. et. al. (1983) 'Acquired immunodeficiency syndrome in male prisoners: new insights into an emerging syndrome'. Ann. Int. Med. 98 p. 297.

8) Pinching A.J. et. al. (1983) 'Studies of cellular immunity in male homosexuals in London.' Lancet ii p. 126.

9) Lane H.C. et. al. (1983) 'Abnormalities of B cell activation and immunoregulation in patients with the acquired immuno-deficiency syndrome'. N. Engl. J. Med. 309 p. 453

10) Buimovici - Klein E et. al. (1983) 'Is the presence of interferon predictive for AIDS?' Lancet ii p. 344.

11) Abbott S.R. et. al. (1984) 'Rapid detection of immunoreactive interferon-alpha in AIDS'. Lancet i p. 564.

12. Penttinnen K. (1977) 'The platelet aggregation test' Ann. Rheum. Dis. 36 supplement p. 55.

13. Essex M. et. al. (1983) 'Antibodies to cell membrane antigens associated with human T cell leukaemia virus in patients with AIDS.' Science 220 859.

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(a) Personal support of applicant(s)

Name	Grade	1st year £		2nd year £		3rd year £	
		Salary	25%	Salary	25%	Salary	25%
T.B. WALLINGTON	CONSULTANT	nil	nil	nil	nil	nil	nil

(b) Research staff (medical/scientific)

Name	Grade	1st year £		2nd year £		3rd year £	
		Salary	25%	Salary	25%	Salary	25%
1. To be appointed	Medical Registrar (Research)	9456	2364	9932	2483	nil	nil
2. To be appointed	Medical Assistant (6 sessions weekly)	3949	987	3949	987	nil	nil
	Totals	13405	3351	13881	3470	nil	nil

(c) Technical/other assistance

Staff category	Age & Qualifications	Grade	1st year £		2nd year £		3rd year £	
			Salary	25%	Salary	25%	Salary	25%
		Whitley Council						
M.L.S.O.		Junior	4675	1169	4728	1182	nil	nil
M.L.S.O.		Junior	4675	1169	4728	1182	nil	nil
M.L.S.O.		Junior	4675	1169	4728	1182	nil	nil
Secretary		Personal Secretary half time	2120	530	2120	530	nil	nil
		Totals	16145	4037	16304	4076	nil	nil

(d) Visiting senior scientist

Name and country from which he/she would be coming to UK	Present position	Period of visit	Salary, subsistence Air fares £	Support available from other sources (e.g. scientist's own institution)
				79/15

EXPENSES

	1st year £	No 'inflation' allowable for years	
		2nd year £	3rd year £
(e) Materials and consumables			
Anti HBc test	2600	2600	nil
TPHA	nil	nil	nil
Interferon alpha	1620	1620	nil
Circulating immune complexes	300	300	nil
Beta 2- microglobulin	1350	1350	nil
Immunoglobulins	100	100	nil
HTLV	nil	nil	nil
(f) Animals Under 'Purchase', applicants should state for each species: (i) the intended source of supply (cf. Project Grants booklet, page 13 paragraph 31; (ii) the microbiological quality required (where appropriate); (iii) the number required; (iv) the price per animal.	nil	nil	nil
(g) Other expenses			
Travel of medical staff in follow up of positive donors and controls	1000	1000	ni
Total £	6970	6970	n

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(h) APPARATUS	£(including VAT)	£(excluding VAT
1) L R 40 Liquid Nitrogen Refrigerator Union Carbide. U.S.A. from Jencons (Scientific) Limited Cherrycourt Way Industrial Estate Stanbridge Road Leighton Buzzard Beds LU7 8UA Cat. No. H 122/8/65	867.10	754.00
2) L R 40 Inventory Control System Source as (1) Cat. No. H 122/13/65	226.84	197.25
3) As (1)	867.10	754.00
4) As (2)	226.84	197.25
	Total £ 2187.88	1902.50

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