Research Protocol

Title	**ZooTB Study**: Estimating the burden of zoonotic tuberculosis in Southwest England
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Sponsor	University of Bristol
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1 Abstract

Zoonotic tuberculosis (zTB) is a form of tuberculosis (TB) disease in humans caused primarily by infection with *Mycobacterium bovis*, the main cause of TB in cattle. Controlling zTB has now been recognised as essential for global TB elimination and is included in The Global Plan to End TB 2016-2020. Transmission of *M. bovis* from cattle to humans can occur through consumption of contaminated unpasteurised milk, raw meat or through aerosols. zTB is clinically indistinguishable from other forms of TB: however, it is resistant to pyrazinamide, a first-line TB drug. In the UK, zoonotic TB was once endemic until pasteurisation of milk and culling of infected cattle controlled the disease in humans. However, there is evidence that zTB attributable to *M. bovis* infection has been increasing since the early 2000s, simultaneous to increasing bovine TB incidence. Despite this, the critical link with infection rates has not been examined. We will address this gap by conducting a retrospective cohort study in Southwest England, recruiting 160 farmers who are in contact with TB-infected cattle. We have selected to study Southwest England since this region is a bovine TB high risk area, where the prevalence of infected cattle is at a high level. Farmers who consent to participate will be asked to complete a questionnaire, this will capture information on the demographic, behaviours, farming practices and bovine TB burden. To establish if a participating farmer has been exposed to *M. bovis*, a blood sample will be collected and tested for latent TB infection using the interferon gamma release assay (IGRA) by collaborators at North Bristol NHS Trust laboratories. IGRA positivity will be used to estimate zTB prevalence in farmers. Questionnaire data will be linked to IGRA results to provide information on potential risk factors for zTB. Data generated from this study will provide key evidence for understanding the current situation of zoonotic TB in a group of individuals who may be at increased risk of exposure due to occupation and/or lifestyle factors. Consequently, this study has the potential to make a significant contribution towards enhancing our understanding of zTB and the implications for public and bovine health.

2 Study Summary

Study Title	Estimating the burden of zoonotic tuberculosis in Southwest England	
Short Title	ZooTB Study	
Study Design	Retrospective cohort study	
Study Participants	Persons in Southwest England who are in contact with cattle	
Planned Sample Size	160 participants (90% participation of 148 farmer pool)	
Planned Study Period	Recruit participants (September 2021 to November 2023) Sample collection and questionnaire deployment (October 2021 to November 2023) Perform immunology assays (by November 2023) Analyse immunological and questionnaire data (by January 2024)	
	Objective	**Outcome Measures**
Primary	Estimate the prevalence of latent TB infection (LTBI) in persons in contact with (TB-infected) cattle.	Collect sufficient samples and participant data to test the hypothesis that persons exposed to TB-infected cattle are more likely to have LTBI.
Secondary	Explore the relationship between a positive IGRA (latent TB infection) and risk factors.	Collect sufficient data on participant demographic and behaviors and link these data link to IGRA test results for determining risk factors for latent TB infection in exposed individuals.
Inclusion Criteria	Individuals will have active involvement with confirmed TB-infected cattle – either through working on or owning a registered cattle holding, or working with cattle located in Southwest England (Avon, Cornwall, Devonshire, Dorset, Gloucestershire, Isles of Scilly, Somerset and Wiltshire) Individuals will be at least 18 years of age.	
Exclusion Criteria	Individuals who have a history of TB disease or in close contacts. Individuals who have symptoms of TB disease: persistent cough (more than three weeks); coughing up blood at any time; fever; night sweats; unexplained weight loss; loss of appetite; swelling of one or more glands in the neck; extreme fatigue and tiredness. Individuals whose occupation or recreational habits do not expose them to cattle. Individuals under 18 years of age. Individuals who fail to provide GP details for the purposes of reporting IGRA test results. Individuals who work with cattle that have not undergone a confirmed bovine TB breakdown.	

3 Background and scientific justification for research

3.1 Zoonotic tuberculosis: natural history, diagnosis and treatment

Zoonotic tuberculosis is a form of tuberculosis disease in humans caused primarily by infection with *Mycobacterium bovis*, the main cause of TB in cattle. Other members of the *Mycobacterium tuberculosis* complex that cause zTB include *M. orygis*, *M. caprae*, *M. microti*, *M. mungi* and *M. pinnipedii* (1). Transmission of *M. bovis* to humans from cattle can occur through consumption of contaminated unpasteurised milk, raw meat or through aerosols.

TB is a complex disease and linking trends in incidence to transmission is difficult because of its natural history. After initial exposure, ~25% of individuals will become infected, with ~10% progressing to active disease: symptomatic (e.g., cough, chest pain, weakness, fatigue, weight loss, chills, fever and night sweats) and identified by culture, microscopy, chest x-ray, molecular assay or by measurable immune response (skin or blood test). The remaining ~90% of infected individuals become latently infected: they have no symptoms, are not infectious and can be detected through measurable immune response. However, a small proportion (~10%) of latent infections can become reactivated later in life and progress into active disease (2). zTB is resistant to pyrazinamide and multidrug resistant strains of *M. bovis* (resistance to at least isoniazid and rifampicin, first line TB drugs) have been identified (1). Therefore, extended treatment is recommended for 9 months, instead of six.

3.2 Tuberculosis epidemiology: globally, the UK and Southwest England

Zoonotic TB may be considered as a neglected tropical disease. Cases predominate in low-income countries and minority and migrant populations within high-income countries (3). Communities at risk of zTB tend to be rural, isolated populations, underserved by healthcare and cases are often not recorded. TB infected livestock herds are common in Europe, Canada, the United States, New Zealand, Africa, Asia, the Middle East, Latin America and Mexico (4). Few countries are free of *M. bovis* (Australia, Iceland, Greenland, Singapore, some European nations, Israel).

In the UK, zoonotic TB was once endemic and transmitted largely by the consumption of raw cows' milk. After the 1960's, pasteurisation of milk and culling of infected cattle controlled the disease in humans; progressively fewer cases are expected to be observed in the older population (≥65 years). However, since the early 2000s cases have been increasing alongside increasing incidence of *M. bovis* infection in cattle. Between 2002-2014, 357 culture-confirmed cases of *M. bovis* disease in humans were identified with an average of 30 cases of zoonotic TB annually (5). The proportion of cases over this period in the UK-born population were increased for younger individuals, suggestive of recent exposure post introduction of pasteurisation. Moreover, it is estimated that the strongest risk factor for human *M. bovis* infection is working in an agricultural or animal-related occupation (adjusted odds ratio 29.5, 95% CI: 16.9–51.6) (5). Despite this, the link between bovine TB and human TB infection has had little investigation in the UK.

3.3 Estimating the burden of zoonotic TB

A human case of active TB can be confirmed using culture, usually of sputum. If it is zTB the isolate will be speciated as *M. bovis*, if it is hTB, it will be speciated as *M. tuberculosis.*
A molecular technique called PCR may also be used and isolates genotyped.
Latent TB cases may be identified by detecting immunoreactivity (an immune response) to *Mycobacterium*. Latent TB means that you have previously been exposed to *Mycobacterium*

but your immune system controlled the infection so that it is dormant. To identify latent TB a blood sample is taken and exposed to different parts of the *M. tuberculosis* bacterium, in particular, components which trigger the immune system, known as antigens. This stimulates the persons white blood cells (T-cells) to produce a cytokine called IFN-gamma, which is then used as a marker of positivity. However, it is not possible to differentiate between *M. bovis* or *M. tuberculosis* using standard diagnostic tests because the same antigens are present on both bacterial species. Nonetheless, blood-based tests for latent TB are a reliable and useful proxy for investigating previous *Mycobacterium* infection.

3.4 Surveillance in the UK

Enhanced TB surveillance in England and Wales was launched in 1999 and provides detailed information of the epidemiology of TB. In 2008 the enhanced Tuberculosis Surveillance (ETS) system was rolled out across the UK. The ETS collects data in real-time on notified cases at clinic, regional and national level (6). Southwest England is a TB low incidence area largely reflective of its socio-demographic characteristics – Bristol is the only local authority with an annual incidence higher than the national rate. Cases are detected as a combination of GP referrals and routine contact investigation.
In 2015, a new migrant Latent TB infection testing, and treatment services was launched in areas with high TB incidence (>20 cases per 100,000); Bristol is the only region in the Southwest to meet this criterion. The majority of agricultural workers in Southwest England live in rural areas and will not be eligible for LTBI testing delivered through primary care. Consequently, cases of zoonotic TB among farmers if detected will be likely be active TB. Our study will therefore help estimate the prevalence of infection in this group of individuals, where estimates could be currently underreported or missed.

3.5 ZooTB study: estimating the burden of zoonotic TB in Southwest England

Controlling zTB has now been recognised as essential for global TB elimination and is included in The Global Plan to End TB 2016-2020 (2). However, much remains unknown: from an accurate assessment of burden to its natural history and clinical outcomes, to a fully articulated impact assessment (3). The ZooTB study aims to estimate the prevalence of zTB in individuals who may be at increased risk of infection due to exposure to TB-infected cattle. Given that the Southwest of England is a bovine TB high risk area, we will be able to readily access farmers who are in frequent contact with TB-infected cattle. Moreover, the diversity of farms in this region (dairy, beef, mixed) offers opportunity to explore a range of risk factors for transmission which relate to farming practices. By collaborating with North Bristol Trust NHS, we are able to deploy standard Public Health England TB services for latent TB testing. Therefore, we are poised to support individuals who otherwise would not have been tested but may be latent TB positive through linkage to standard NHS support and guidance. Finally, Bacillus Calmette-Guérin (BCG) is the only licensed vaccine against tuberculosis. In 2005, universal vaccination of school-aged children in England stopped and policy changed to only targeted vaccination of high risk neonates (7). Consequently, studying zTB infection in a population who may be unvaccinated, or who have unvaccinated contacts e.g., children, has potential to impact on policy in high risk individuals. Data generated in this study will help scientists and public health officials understand the burden of zTB in the UK, in turn informing effective public health and vaccination programmes, as well as improve dialogue and efforts to control bovine TB.

4 Lay Summary

Zoonotic tuberculosis (zTB) is a form of TB disease in humans caused mainly by infection with the bacterium *Mycobacterium bovis*, the main cause of TB in cattle. zTB is considered a

neglected disease and prevalence is likely underestimated due to poor surveillance and difficulties in diagnosis. *M. bovis* can be transmitted from infected cattle to humans by consumption of contaminated raw milk and meat, or via aerosols. Southwest England is at the centre of the bovine TB (bTB) epidemic. Therefore, individuals in close contact with bTB may be at increased risk of exposure. One method to assess whether someone has been exposed to *M. bovis* and is latently infected is by measuring a person's immune response to the bacterium using a blood-based test. We will use this test to determine the prevalence of zTB in persons in contact with TB-infected cattle in Southwest England. We will ask participants to complete a short questionnaire about themselves and their contact with TB-infected cattle. We will use this information in conjunction with blood test results to explore what risk factors, if any, are associated with becoming infected with bovine TB. We will analyse the results and publish them in a scientific journal, and we will engage with the farming community directly. This study will help provide an estimate for zTB prevalence and risk factors in a bovine TB high risk area, providing essential evidence to inform science and policy relating to TB control in both cattle and humans.

5 Principle Research Question

What is the prevalence of zTB in persons in contact with cattle in Southwest England and what risk factors, if any, are associated with a positive IGRA in this population?

6 Objectives and Outcome Measures

Primary Objective: Estimate the prevalence of latent TB infection (LTBI) in persons in contact with (TB-infected) cattle
Secondary Objective: Explore the relationship between a positive IGRA (latent TB infection) and risk factors.

7 Summary of Design and Methodology

The ZooTB study is a retrospective cohort study of farmers in Southwest England. This demographic may be at increased risk of occupational exposure to TB-infected cattle, particularly so in this region, with ~50% of herds having been infected with TB since 2000. Farmers who consent to participate will be asked to complete an electronic questionnaire and provide a blood sample. We will host study test days at agricultural shows and at research study events, which may include local veterinary practices. Blood samples will be collected at study test days by a North Bristol NHS Trust phlebotomist.
Blood samples will be used to measure latent TB infection (LTBI) using the IGRA, as a way to measure cattle-to-human TB transmission. IGRA positivity will be used to estimate the prevalence of LTBI in persons in contact with cattle.
The questionnaire will collect information on demographic and health data (farmer and cattle); consumption of raw milk and milk products; bovine TB burden; farming practices and behaviours. Questionnaire data will be linked to IGRA results to identify risk factors for LTBI.

8 Participant Recruitment Criteria

8.1 Inclusion Criteria

Individuals will have active involvement with confirmed TB-infected cattle – either through working on or owning a registered cattle holding, or working with cattle located in Southwest England (Avon, Cornwall, Devonshire, Dorset, Gloucestershire, Isles of Scilly, Somerset and Wiltshire)
Individuals will be at least 18 years of age.

8.2 Exclusion Criteria

Individuals who have a history of TB disease or in close contacts.
Individuals who have symptoms of TB disease: persistent cough (more than three weeks); coughing up blood at any time; fever; night sweats; unexplained weight loss; loss of appetite; swelling of one or more glands in the neck; extreme fatigue and tiredness.
Individuals whose occupation or recreational habits do not expose them to cattle.
Individuals under 18 years of age.
Individuals who fail to provide GP details for the purposes of reporting IGRA test results.
Individuals who work with cattle that have not undergone a confirmed bovine TB breakdown.

9 Study Procedures

9.1 Patient and Public Involvement

9.1.1 Design of research

We have conducted four public involvement groups related to this study: firstly, with a small group of invited farmers in Taunton recruited via Synergy Veterinary Practice; with 26 farmers at a clients evening at Langford Vets; at Southwest Livestock markets; and online.

All of the farmers in the first group reported drinking unpasteurised milk from the bulk milk tank and felt that it was protective against infections. Many had had experience of zoonotic infections; one reported that they had contracted Brucellosis and that the local GP had not diagnosed it. All farmers thought that their children and grandchildren should be eligible for the BCG vaccine. One farmer had tried and failed to get his children vaccinated privately.

In the Langford Vets' clients evening, we gave a presentation with discussion to 26 farmers and partners. In a poll, 24 out of the 26 farmers said that they would agree to being tested for latent TB as part of a study.

At Frome Livestock Market farmers who attended the market said they would agree to being tested and complete a questionnaire if a study day was held at the market.

We conducted a one-hour online public involvement session with four farmers. Topics included: what TB means to them; their TB experiences; current engagement with zoonotic TB; study feasibility and practicalities. Discussion led to co-designing optimal strategies for hosting study test days and questionnaire completion. Participants were very keen on agricultural shows as sites for hosting test days and felt that questionnaire completion could either be electronic (remotely), or simultaneous to providing a sample with a researcher (electronic or paper). All farmers agreed that the research topic was important and had potential to raise the profile of bovine TB control. All participants felt interest in the topic was motivation enough to participate in the study and would be willing to travel a reasonable

distance to do so. Participants raised concerns that true TB free herds would be very difficult to find and some contact with TB infected cattle by most farmers was likely.

9.1.2 Management, undertaking, analysis and dissemination of research

We have been awarded funding to complete a research project titled 'Engaging the farming community in zoonotic disease research' from the NIHR Health Protection Research Unit in Behavioural Science and Evaluation Community Involvement Scheme). The project will be conducted alongside The ZooTB study. It aims to embed representatives from the farming community in zoonotic TB research, and through their involvement, co-develop the study throughout all stages. In turn we will gain approval of the farming community for our study design, methods of research and dissemination of results.
We have recruited two farmers who we meet with monthly. The farmers are invited to co-create study documentation and study design and have been invited to attend sampling days. Through their involvement we will improve communication between farmers, the scientific community and policy makers, as well receiving major contributions towards co-developing the study with representatives from the target population.

9.2 Participant Information Sheets

We are aiming to invite and enrol the majority of participants prior to blood sampling conducted at agricultural shows. However, prospective participants at agricultural shows who do not yet know of the study and are interested to take part will have the opportunity to enrol. To accommodate these two methods of recruitment, invitation and participant information sheets will be distributed as follows: -

i. The study is promoted to potential participants online (study websites and social media), via email/newsletter, in-person or via letter using recruitment leaflets. Individuals who express an interest to take part in the study are emailed an invitation to participate or can request a call back from a member of the study team for further information. The invitation email will contain an electronic Participant Information Sheet (PIS) as a pdf attachment, as well as a hyperlink to the REDCAP e-consent module. In particular, the PIS includes contact details of the research team, giving potential participants the option to ask questions about and discuss any aspect of the study.
ii. At study test days hosted at agricultural shows or research study events, prospective participants can meet the study team and enrol on the day. Participants will be given a paper PIS and will have the option to ask questions and discuss any aspect of the study with the research team on the day.

A copy of the Participant Information Sheet is included in the Appendix.

9.3 Informed Consent

Following distribution of the Participant Information Sheets, informed consent will be collected using an e-consent webtool, hosted securely on a REDCap database, which will help limit physical contact in line with current pandemic guidance by the Health Research Authority (8). Consent specifically addresses issues pertaining to storage and sharing of samples and data, and the possibility of re-contacting participants for any follow-up studies. The invitation email and PIS will contain a hyperlink that directs individuals who are interested in participating to the e-consent webtool. This will be accessible from any internet-enabled mobile or desktop device. If consent is collected at the agricultural show, the e-consent webtool is able to work off-line on the REDCap app hosted on a study tablet. A copy of the consent script is given in the Appendix.

9.4 Enrolment into Study

Directly after consenting to take part using the REDCap consent tool, participants will be:

1) Asked to provide contact details: telephone/mobile phone number, home email address and residential address. Phone number and email will be used to deliver i) a reminder of their scheduled blood sampling appointment at the agricultural show and ii) to complete the online questionnaire and iii) enable relaying IGRA test results.
2) Allocated a unique study ID number that will accompany the participants questionnaire and blood sample
3) Be directed to the online questionnaire

9.5 Study questionnaire

Participants will be asked to complete a short questionnaire capturing information on demographic, behaviours, farming practices and bovine TB burden (given in Appendix). The questionnaire will take approximately 15 minutes to complete and will be conducted electronically. Participants will be provided with a link to complete the questionnaire after they have e-consented. The questionnaire will be hosted securely using REDCap.

If participants have been unable to complete the questionnaire online or they are enrolling on the day of the agricultural show, the questionnaire can be completed using REDCap on a study tablet.

9.6 Blood Sampling

Following enrolment, participants will be contacted by a study team researcher to organise an appointment for blood sampling at a study test day. Participants will receive a SMS/email reminder the day prior to testing. Venepuncture will be performed by an appropriately trained clinical delegate from North Bristol NHS Trust at the study sites (agricultural shows or research events) and/or an appropriately trained individual with a university contract.

Blood sampling will involve peripheral venepuncture to obtain approximately 4mL blood in total, collected into 4 separate tubes for the interferon gamma release assay. An optional extra 5mL blood sample will be collected into a single serum-separating tube (SST) for future infection and immunity research. A butterfly needle closed vacutainer system will be used. Blood samples will be labelled immediately following the procedure by the practitioner performing the procedure. Following standard NBT venous sampling protocol, the venous blood samples will be labelled as follows: full name, date of birth, zooTB study ID number, date and time of sampling, member of staff's signature or initial.

Samples will be placed within a clear plastic resealable bag and kept at room temperature in a designated box. Blood samples will be transported to the North Bristol Trust NHS laboratory according to their standard operating procedures for biological specimens.

9.7 Benefits for Participants

From our PPI sessions we learnt that farmers were highly engaged in the research area and were willing to take part in the study because they believed it was valuable research to their community, as well as having the potential to contribute towards tackling bovine TB.
For individual participants, they will have the benefit of finding out their latent TB status. This would have no implications for their current health status, but if they should develop any TB-

like symptoms in the future, they will have had discussions with their GP and possibly a member of their local TB service to help facilitate treatment if deemed clinically necessary. Farmers will have the opportunity to engage with scientists and public health officials to gain knowledge on zTB. Data generated has the potential to impact on policies surrounding BCG vaccination and bovine TB control.

9.8 Risks and Burdens for Participants

Consent and data collection will be performed using a secure online platform (REDCap) to avoid unnecessary physical contact between participants and researchers. Where consent and questionnaires are completed at the study site, the study will conform to UK Government guidance on physical distancing and Health Research Authority guidelines on performing research during the COVID-19 pandemic (8,9).

Risks of venepuncture include: i) fainting or feeling light-headed: all venepuncture will be performed sitting down; ii) minor hematoma/bruise: this will be minimised by the application of pressure at the site of the venepuncture using gauze for 3 minutes following the procedure. Venepuncture will only be carried out by appropriately trained professionals. First aid and ambulance teams at study sites (agricultural shows) will be informed of the study and will be available to provide support if required.

10 Laboratory Procedures

10.1 Biosafety

10.1.1 Blood sampling

Blood sampling will be conducted by North Bristol NHS Trust personnel under their standard operating procedures. Venepuncture will be undertaken using a closed vacutainer system to minimise the risk of blood spillage. Blood samples will be placed within a tube rack, upright, and kept at room temperature in a designated sample transport box.

10.1.2 Transport of samples from participant testing site to research laboratory

Blood samples will be transported to North Bristol NHS Trust TB laboratories for analysis according to North Bristol Trust standard operating procedures.

10.1.3 Laboratory biosafety: blood samples

Samples will be processed according to standard operating procedures established for conduct of IGRA at North Bristol NHS Trust TB laboratories. See Appendix for laboratory protocol.

10.2 Evidence of latent tuberculosis infection

Blood samples will be tested for evidence of latent tuberculosis infection using the Interferon Gamma Release Assay (QuantiFERON-TB Gold, Qiagen) according to standard operating procedures at North Bristol NHS Trust laboratories (see Appendix). The QuantiFERON system uses specialised blood collection tubes. Blood is collected into four tubes: a Nil tube, x2 TB antigen tubes, and a mitogen tube. Whole blood collected is incubated within 16 hours of collection. Incubation occurs in the tubes for 16 to 24 hours at 37°C, after which, plasma is harvested and assayed for the presence of IFN-gamma produced in response to the peptide antigens. IFN-gamma is measured by ELISA (enzyme linked immunosorbent assay)

and expressed as IU/ml. A test is considered positive for an IFN-γ response to the TB Antigen tube that is significantly above the Nil IFN-γ IU/ml value.

10.3 Supplemental work

In the e-consent script, consent is specifically sought from participants to perform supplemental analyses that enriches the exploration of our primary research question. However, blood collected will be destroyed within 1 week of collection after laboratory analysis is complete. If participants consent for an optional extra blood sample to be used in future research, it will be donated to Bristol Biobank (https://directory.biobankinguk.org/Profile/Biobank/GBR-1-112) so that it can be used for further research relating to infection and immunity.

10.4 Future work

We can only consider further studies (and what samples might be required) once we have all the results of the current study. Therefore, we ask specifically in the consent form for permission to contact participants in the future to inform them about follow-up studies that they might be interested in (which would require separate REC approval).

11 Timeline of Investigation

Trends in the COVID-19 pandemic are under continual observation and any restrictions imposed during the study timelines will be adhered to. Consequently, study timelines may be disrupted and adjusted in response to government restrictions.
Two key events in the farming calendar have been identified as potential study test days: -

1. The Dairy Show, 6th October 2021, The Royal Bath and West Showground (https://www.bathandwest.com/the-dairy-show)
2. Royal Cornwall Show, 9-11th June 2022 (http://www.royalcornwallshow.org)

These events are expected to be very well attended by prospective participants. The first study day will be used as a pilot study. Results will be used to refine sample size calculations, refine procedures, and be used to promote the study for strengthening recruitment at the subsequent test days. Study timelines are presented in Table 1.

Table 1: Study timeline

Study Design Month -6 to 0	Recruitment Month 1 to 2	Data and sample collection Month 3 to 11	Laboratory assays Month 3 to 11	Analysis and dissemination Month 12 to 18
- Patient public involvement and collaborative study design	- Email invitation with Participant Information Sheet - Discuss any participant questions as necessary - E-consent	- Electronic questionnaire - Blood sample at agricultural show	- Interferon gamma release assay (IGRA) performed at North Bristol Trust NHS laboratory	- Analysis of questionnaire data and linkage to IGRA results - Write-up of report - Dissemination of results to and engagement with the farming community - Submission to journal

12 Rationale for study population, procedures and environment

The Southwest of England is the centre of the bovine TB epidemic in cattle and has the highest zTB rates per capita. Despite this, the critical link with infection rates has not been examined. Therefore, farmers exposed to TB infected cattle in the Southwest are well suited as a population to study for estimating the burden of zoonotic TB in this high-risk region of the UK.

13 Specific Ethical Considerations

This is an observational study and there are few study-specific ethical implications, outside of confidentiality and data protection (governed by GDPR) and the handling and storage of samples (governed by the Human Tissue Act and Health & Safety Executive).
The study has been designed in participation with representatives from the farming community. Participant recruitment and informed consent will take place in accordance with the principles of the Declaration of Helsinki.

Blood test results for LTBI will be fed back to participants by a member of the study team according to their preferred method of communication, e.g., email or letter, along with an information sheet. As study participants would not usually receive a LTBI test we have developed a protocol in the event of a positive or indeterminate interferon gamma release assay (IGRA). If an individuals IGRA result is positive or indeterminate, the participants GP will be informed and given the relevant information sheet. For positive results, we suggest that the GP refers the participant to a local hospital for a non-urgent discussion. For indeterminate results, we advise GPs to discuss this result with the participant, including discussion of possible TB symptoms to look out for and action to take if symptoms arise. If the participant has any concerns about their health or that of their family or colleagues, then we suggest they should consult their GP in the usual way. Dr Ed Moran will be available to

support GPs should they have any clinical queries and also for support to local TB clinical services should participants be referred to them.

14 Statistical considerations

Study design
This is a retrospective cohort study. We will first conduct a pilot study at The Dairy Show in October 2021. Results obtained will be used to refine sample size calculations to determine prevalence and risk factors for LTBI in those exposed to TB-infected cattle in a larger cohort.

Primary outcome: Positive Quantiferon TB test, defined as using the standard cut-off of 0.35 IU/ml (international units/millilitre).

Estimated effect size: There are no available surveys of LTBI in persons exposed to *M. bovis* in the UK. TB incidence in Southwest England is as low as 5.3 per 100,000 persons with estimated LTBI prevalence less than 1%, evidenced through routine contact investigation. In Southwest England, ~5 *M. bovis* TB cases are diagnosed per year, therefore 25 cases have progressed to disease from LTBI in the past 5 years (primary progression). Based on PHE data, 50% of cases are laboratory-confirmed and from knowledge of TB immunology up to 10% of LTBI cases develop symptoms within 5 years of exposure. Therefore, ~500 (=25x2x10) people have LTBI infection due to *M. bovis* in Southwest England. From Defra data, ~4,000 cattle herds are Officially Not TB Free in Southwest England. Testing one person per herd, LTBI prevalence could be 12.5% (=100x500/4000).

Sample size calculations
Pilot sample size
Using a binomial distribution, the probability of obtaining zero positive samples is $(1 - prevalence)^N$, where N is the sample size. If we want this probability to be greater than 1% (greater than background prevalence of LTBI attributable to *M. tuberculosis*), then the required sample size will be $N > log_{10}(0.01)/log_{10}(1 - prevalence)$, which equals 34.5, i.e., at least 35 samples.

With a sample of 35 and a true prevalence of 12.5%, we would expect to obtain between 1 and 9 positive samples on 95% of occasions, corresponding to a 95% confidence interval for the prevalence between 0.15% and 43.6%.

A pilot study was conducted at The Dairy Show in October 2021 and recruited 26 farmers. All participants completed the questionnaire and provided a blood sample for IGRA. Of these, no individuals were latent TB positive (0/26, 95% CI: 0-16.0%). Although we did not achieve the intended pilot sample size of 35, our expected prevalence of 12.5% is within the confidence interval for the result obtained. Therefore, we will proceed with no refinement to the sample size calculation for the full study.

Sample size for full study
For the full study, to estimate the risk of transmission by exposure level (i.e., raw milk consumption and exposure to infected cattle), we assume prevalence to be 20% in highly exposed individuals, vs 5% in the low-level exposure group. Using a two-sample comparison of proportions with 80% power and 5% significance level (power.prop.test(p1=0.05,p2=0.2,power=0.8, sig.level=0.05) in R) gives 76 samples per group. Simulating such a study 10,000 times by sampling 80 times from binomial distributions with means of 0.05 and 0.2 and using Fischer's exact test to calculate the p-

value, gives a p-value of less than 0.05 on 80% of occasions. Therefore, we will aim to test 160 people in total in the first instance.
After completing the pilot study, we will refine sample size calculations for the full study.

Statistical analysis
A positive IGRA will be used to estimate zTB prevalence in persons exposed to TB infected cattle. Prevalence will be reported alongside confidence intervals for a single proportion calculated using the Wilson method.

A logistic regression model will be used to explore the relationship between a positive IGRA and exposure level to *Mycobacterium bovis,* with exposure comprising differing levels of contact with bTB infected cattle and raw milk products. Behaviour change associated with TB infected cattle will be reported qualitatively.

Data obtained in the pilot and full study will be analysed together. Possible differences in effect size due to recruitment occurring in multiple waves (pilot and full study) will be adjusted for by including recruitment wave as a covariate in statistical models. Characteristics of different recruitment waves will be monitored for differences that could inhibit analysis as one dataset.

This is an exploratory study and any evidence gathered on the link between cattle, humans and TB infection will be valuable data.

15 Confidentiality and data protection

15.1 Storage and Use of Personal Data

Data will be collected and retained in accordance with the Data Protection Act 1998. The study team will be responsible for data collection, recording and quality control. Study documents containing details of demographic data, documentation of inclusion and exclusion criteria, and medical history will be retained for a period of 10 years following the end of the study.

15.2 Confidentiality

Study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participant number. All documents will be stored securely and will only be accessible to study staff and authorised personnel. The study will comply with the General Data Protection Regulation (GDPR) which requires data to be anonymised as soon as it is practical to do so.

15.3 Access to Data

The Chief Investigator will allow monitors (on behalf of the Sponsor), persons responsible for the audit, and representatives of the Regulatory Authorities to have direct access to source data/documents. This is reflected in the Participant Information Sheet (PIS). Study monitoring will be undertaken on behalf of the Sponsor by University Hospitals Bristol and Weston NHS Foundation Trust using their monitoring standard operating procedure. Access to study documents will only be available to the investigators, monitors and auditors directly involved in the study. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/initials, not by name.

15.4 Notification of Other Health Professionals of Participant Involvement

Study participants would not usually receive a LTBI test, so we have developed a protocol in the event of a positive or indeterminate IGRA. An individual's IGRA result (positive, negative or indeterminate) will be relayed to them directly by a member of the study team according to their preferred method of communication e.g., email or letter. If an individual's IGRA result is positive or indeterminate, the participants GP will be informed. Participants who do not share their GP details at the consent stage will not be eligible to enrol into the study. The GP will be advised to discuss the result with the participant, including which symptoms to look out for following indeterminate or positive results. We suggest that the GP refers the participant to a local hospital for a non-urgent discussion regarding the positive IGRA result. If the participant has any concerns about their health or that of their family or colleagues, then we suggest they should consult their GP in the usual way. Dr Ed Moran will be available to support GPs should they have any clinical queries and also for support to local clinical services should participants be referred to them.

16 Use of human tissue

Due to the IGRA procedure as outlined in 10.2 above, blood samples are collected into 4 specialised tubes. The blood sample is incubated within 16 hours of collection. Following completion of the assay the human tissue will be destroyed (usually within one week of collection), or if participants consent for an optional extra blood sample to be used in future research, it will be processed for serum and donated to Bristol Biobank (https://directory.biobankinguk.org/Profile/Biobank/GBR-1-112) so that it can be used for further research relating to infection and immunity. When assays have been conducted on all samples, the Chief and Principal Investigators will confirm with NBT that all biological samples have been destroyed.

17 Safety

This study conforms to UK Government guidance on physical distancing and Health Research Authority guidelines on performing research during the COVID-19 pandemic (8,9). Consent and questionnaire completion are being performed using a secure online platform to avoid unnecessary physical contact between participants and researchers.

Venepuncture will only be carried out by appropriately trained professionals in accordance with North Bristol Trust NHS standard operating procedures. The procedures are relatively safe for the participant. Risks of venepuncture include: i) fainting or feeling light-headed: all venepuncture will be performed on an examination couch; and ii) minor hematoma/bruise: this will be minimised by the application of pressure at the site of the venepuncture using gauze for 3 minutes following the procedure.

18 Indemnity

The University of Bristol has arranged Public Liability insurance to cover the legal liability of the University as Research Sponsor in the eventuality of harm to a research participant arising from management of the research by the University. The University of Bristol holds Professional Negligence insurance to cover the legal liability of the University, for harm to participants arising from the design of the research, where the research protocol was designed by the University. The University of Bristol's Public Liability insurance policy provides an indemnity to our employees for their potential liability for harm to participants during the conduct of the research. In addition, investigators have the protection of medical malpractice indemnity with the Medical Protection Society or Medical Defence Union.

19 Reporting and Dissemination of Results

The results of the study will be published in appropriate peer-reviewed academic scientific journals and will be presented at scientific conferences. No identifiable personal data will be published, all data will be completely anonymised. At the end of the study, a final anonymised dataset will be made available for third party researchers through a suitable data repository alongside code to reproduce statistical analysis. Results from the IGRA will be reported to participants in line with protocols developed and described in 15.4 above. IGRA results will be reported to participants in the form of a letter or email, alongside an information sheet specific to the result (positive, negative or indeterminate). A lay summary of findings will be co-produced with members of the farming community for dissemination at the end of the study. This will be shared with participants and will be visible on the study website.

20 Potential Impact

The ZooTB study will address our gap in understanding the burden of zoonotic TB in individuals in contact with TB infected cattle in Southwest England. The study will provide an estimate of prevalence in this potentially at-risk demographic and explore risk factors for exposure. Data generated will help scientists, public health officials and policy makers understand the evidence for this neglected disease in an under studied population. This has the potential to impact on TB control in both animals and humans: at the animal-interface measures to control bovine TB will need to be given greater attention if there is evidence of transmission to humans; at the human-interface, policy changes such as BCG vaccination of individuals in contact with cattle could be implemented to help reduce infection. Moreover, reducing the burden of bovine TB will positively impact on the mental health and wellbeing of farmers who have been at its peril since it emerged.

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22 Appendix

22.1 Venepuncture policy North Bristol NHS Trust

NHS North Bristol NHS Trust

Venepuncture Policy

CP1c

Specific staff groups to whom this policy directly applies	Likely frequency of use	Other staff who may need to be familiar with policy
All staff who undertake Venepuncture Staff who manage those undertaking venepuncture	Often	Heads of Nursing/Heads of Clinical Divisions

Main Author(s):	Mooi Tay
Consultation:	Phlebotomists Laboratory manager Staff Development Health and Safety Infection Control & Prevention Nurse Renal Dialysis Sister
Ratifying Committee:	Clinical Effectiveness Committee
Date Ratified:	January 2019
Review Date:	January 2021
Version:	6
KEYWORDS:	Venepuncture

Page **1** of **24**

Venepuncture Policy		
Document Status:		
Version	**Date**	**Comments / Summary of Change**
1	May 2002	Developed and approved by N&M Policy and Practice Committee
2	June 2005	Reviewed – no changes
3	June 2008	Reviewed – Blood Cultures Policy removed
4	December 2012	Reviewed – Infection Control and Prevention updates, amendments made to include paediatric specific practices.
5	December 2015	Reviewed – Updated to include safer sharps practice, policy updates. Section 7 added to highlight specific practice
6	October 2018	Reviewed – Date and time of venepuncture must be reflected on the label and request from, practices in NICU, amended competency assessment form

NHS North Bristol NHS Trust

Venepuncture Policy

Executive Summary

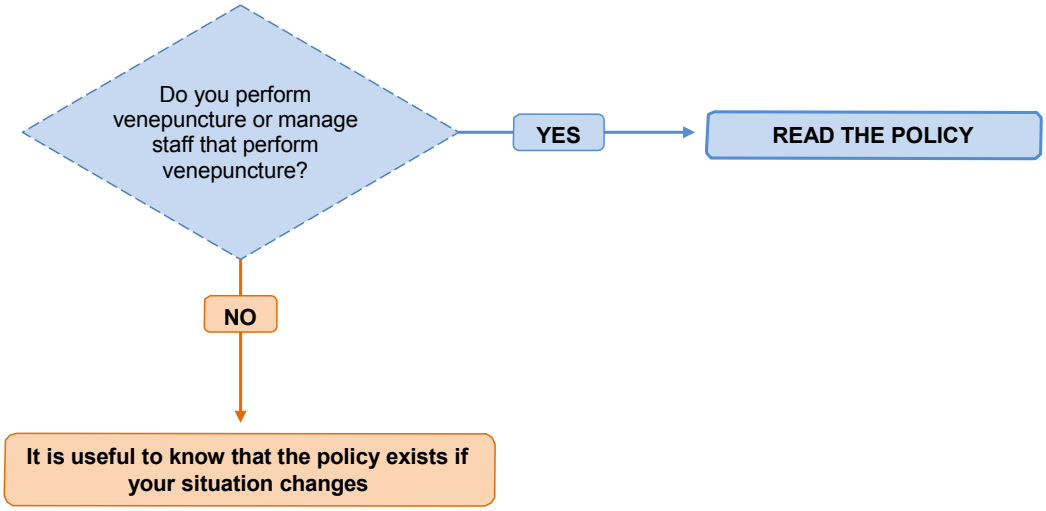
This Policy will provide information about the correct technique used for venepuncture within North Bristol NHS Trust. By using this policy the user will act to reduce the risks to patients and practitioners associated with the venepuncture procedure.

Summary of key points

- This Policy will apply to all practitioners who undertake venepuncture as part of their role.
- All practitioners who are able to perform venepuncture are accountable for their practice and are responsible for maintaining their competence in this skill.
- Patients must be correctly identified prior to blood sampling taking place.
- Sample tubes must be labelled at the patient bedside immediately following collection by the practitioner who carried out the procedure.

Venepuncture Policy

DO I NEED TO READ THIS POLICY?



Contents

NHS North Bristol NHS Trust

Venepuncture Policy

1. Policy Statement

It is the Policy of North Bristol NHS Trust (NBT), that all venepuncture is undertaken in a safe and consistent manner.

2. Purpose of the Policy

This Policy has been developed in order to reduce the risks and complications associated with venepuncture.

3. Scope of the Policy

This Policy applies to all practitioners who are required to undertake venepuncture as part of their role from a patient's upper limbs.

4. Definition of Terms

Competent Assessors - are defined as practitioners who have undergone training, workplace assessment and deemed competent to practice the technique as an integral part of their clinical role.

CVAD - Central Venous Access Device

MLE - Managed Learning Environment

ICE - Integrated Clinical Environment Order Communications System

5. Roles and Responsibilities

5.1 Accountability

- All practitioners who are able to perform venepuncture are accountable for their practice and must ensure they maintain their competence in this skill. They must also make sure that their competency is recorded on the MLE.
- If they do not use this skill for an extended period of time and it remains a requirement of their role, they are required to update their competence appropriately. This could be achieved by undertaking a formal Clinical Skills Update with Staff Development or by means of assessment by a competent practitioner in the clinical area.
- All relevant practitioners must be aware of this Policy and have read it.
- A registered practitioner is accountable for delegation of this skill to an un-registered practitioner.

- It is the responsibility of the ward/clinical area manager to ensure that all relevant practitioners have undergone training and competency assessment and are aware of this Policy.

- Practitioners must demonstrate a professional Duty of Candour at all times so that any errors are reported which would highlight areas for potential change in practice.

- Further training is required to take Blood Culture sample and to take venous blood sample from a lower limb.

5.2 Un-registered Practitioners (Band 2, 3 and 4)

- Un-registered practitioners who have undergone recognised training and assessment of competence are able to perform venepuncture, if clinically indicated. Any problems with the venepuncture procedure, i.e. swelling or haematoma, must be documented and reported to the registered practitioner responsible for the patient.

- Band 2, 3 and 4 practitioners may perform venepuncture as long as this is a skill they will perform regularly and it is in their job description. They may undergo the training if it is part of their training programme. They must make sure that their competency is recorded on the MLE.

5.3 Students

Students who have undertaken recognised venepuncture training may perform venepuncture under the direct supervision of a registered practitioner who is competent in venepuncture.

They can access the training at North Bristol NHS Trust (NBT) upon the approval of their mentor, and arrangement has been made in their placement for them to have supervised practice and competency assessment.

6. The Venepuncture procedure

- Before venepuncture is attempted, valid consent must be gained (Policy CG07: Consent for Examination and Treatment).

- Before venepuncture is attempted the practitioner must identify the patient correctly, by asking the patient to state their full name and date of birth. Their hospital / unique identification number must be checked using the ICE label or sample request form against their two identification wrist bands. For outpatients where wrist bands are not used, other patient records can be used to confirm the hospital / unique identification number against the ICE label or sample request form (Policy CP7g: Patient Identification).

- Where patients are unconscious or unable to identify themselves the practitioner must carry out the checks as above and ask another practitioner or accompanying carer to verify the patient's identity is correct (Policy CP7g: Patient Identification).

- The practitioner must discuss the patient's allergy status, specifically latex allergy, and prior to the procedure. Any identified allergies/adverse reactions must be clearly documented and electronic patient records updated.

- Ensure the patient has met any special requirements for the blood sample, for example, if the patient has fasted.

- If the sample is for ascertaining drug levels, the time of the last dose of the drug must be recorded. This could be completed in the clinical details section on the ICE request.

- Assess the patient for medical conditions which may affect the procedure, for example, haemophilia.
- The practitioner must be aware of any relevant medication the patient is taking, an example is anticoagulant therapy. This must be recorded on ICE.
- Only medical practitioners may undertake venepuncture of the lower limbs in adults. Exceptions to this are phlebotomists and practitioners who have undergone additional training and competency assessment. Access to this training is arranged through the Clinical S kills Team in Staff Development and must be recorded on the MLE.
- A close vacuum system must be used when taking blood within NBT. A needle and syringe must not be used to perform venepuncture. The exceptions to this are in Neonatal care (see Section 7). If a butterfly needle is required, this must also be a vacuumed system. All sharps used for venepuncture must incorporate a needle safety device.
- The blood sample tubes must be taken in the order recommended by the manufacturer or Pathology Laboratory. This is the “order of draw” (See appendix 3).
- After sample collection and disposal of sharps, the tubes must be inverted (NOT shaken) as specified by the Pathology laboratory (see appendix 3) to ensure mixing of blood and preservatives.
- Sample tubes must be labelled by the practitioner carrying out the venepuncture procedure before leaving the patient’s side. The patient must be asked where possible to again state their full name and date of birth. This information, along with hospital / unique identification number, must be checked against their wrist bands and ICE label / request form. If all is correct the sample tube can be labelled or hand written as appropriate.
- Please amend the date and time of collection on the ICE label or on tube if necessary. It is crucial to put in the exact time and date of sampling.
- Two venepuncture attempts may be made, if unsuccessful, assistance must be sought from another competent practitioner. If the blood tests cannot be collected, it must be documented in the patient record and the requesting practitioner must be informed. In an emergency situation, when the priority is obtaining a blood sample, more than two attempts are permitted.
- Needles used in venepuncture are single use items. Needles must be disposed of safely in an approved sharps container at the point of use, in accordance with the Sharps Management policy (HS07).
- Patients can clench their fist to bring up the veins before venipuncture, but, they must not pump their fists.
- For step by step guidelines on how to undertake the venepuncture procedure see appendix 4.
- Obtaining blood from a CVAD must only be performed by a competent staff (see Section 7.0)

6.1 Infection Control

- All practitioners must be 'naked below the elbow' whilst on wards or in other clinical areas. This enables thorough hand washing including the wrist area and prevents continuing carriage of potential pathogens on wrist and hand jewellery.
- All practitioners undertaking venepuncture are advised by Occupational Health to be immunised against Hepatitis B.
- The Needle stick Hotline is 01173423400.
- Hand decontamination must be undertaken prior to venepuncture.
- An aseptic non-touch technique must be used for venepuncture.
- Practitioners performing venepuncture must wear non-sterile gloves. Exceptions to this are when undertaking a difficult venepuncture, when gloves may be removed (however any cuts or lesions must be covered as not wearing gloves increases the risk of infection to the practitioner). Emphasis in these circumstances is on good hand decontamination.
- Disposable plastic aprons must be worn for the procedure.
- The venepuncture site must be thoroughly cleaned with 2% Chlorhexidine in 70% isopropyl alcohol for 30 seconds. It must then be **left to dry**. Povidine Iodine 10% must be used as an alternative if the patient is sensitive to Chlorhexidine. It is recommended that the site is not re-palpated after cleaning, although this may be difficult where the vein is hard to find.
- For all infants, a solution of 2% Chlorhexidine in 70% isopropyl alcohol (ChloroPrep®) must be used in an accepted Neonatal applicator as specified in the NICU Skin cleansing guidance. It must be applied in a dabbing technique and only by practitioners trained in its use due to the terms of the license and the associated risks of chemical burns caused by improper use. The site must be cleaned for 10-20 seconds and not necessarily until the applicator is empty and allowed to dry to a matt finish (minimum of 30 seconds). There may be special circumstances in cases where neonates are extremely preterm (less than 27 weeks gestation) whereby specialist advice must be sought from NICU.
- For all infants, a sterile pack is used and sterile gloves must be worn following appropriate and thorough hand decontamination.
- **All tourniquets must be single use only**. Gloves MUST NOT be used as tourniquets as they are not designed for the purpose. Tourniquets are not to be used in NICU. Tourniquets must not be left on for longer than 1 minute as this has shown to alter some blood results. This also promotes patient comfort.

6.2 Disposal of waste

- All sharps used during venepuncture must be disposed of safely in an approved sharps container, at the point of use, in accordance with the Sharps Management Policy (HS07).
- All waste that has been used during the venepuncture procedure must be disposed of as per the Waste Management Policy (HS29)

6.3 Documentation

- The practitioner performing venepuncture must, whenever reasonably practicable, document the procedure in the appropriate patient record, e.g. patient notes, Lorenzo, or Integrated Clinical Environment Order Communications System (ICE).
- Any difficulties encountered during venepuncture must be recorded within the patient's record.

6.4 Training and Competence

- All practitioners undertaking venepuncture unsupervised must have received appropriate training and deemed competent to do so. They must make sure that their competency is recorded on the MLE.
- See Appendix 2 for the Trust's standardised competency framework for venepuncture. Completion of competency assessment must be recorded on the MLE by Staff Development. There is an expectation that competency assessment will be completed within three months of training. If competence is not achieved within twelve months of attendance at training, practitioners m a y be expected to repeat the training before undertaking assessment of competence.
- It is accepted that there are practitioners who commenced this practice prior to the availability of formal training and assessment, and who practice the skill regularly. These practitioners must, however, ensure that they have read and comply with the current policy and undertake assessment of competence using NBT's competency framework.
- Practitioners entering the Trust who have been trained in other organisations must be able to produce evidence of comparable training. Non-medical practitioners must be able to produce evidence of current competence, and undergo competency assessment in their clinical area using the NBT assessment tool (appendix 2) by another practitioner competent in venepuncture. All practitioners must familiarise themselves with this policy prior to practicing venepuncture.
- Venepuncture training is available through the Staff Development Department. The Clinical Skills Team must be contacted for further information regarding the training and contact details are available via the Trust intranet site (Learning & Development /clinical skills). Check the MLE for the training schedule.
- *Practitioners who are competent in venepuncture and/or central venous access device management will automatically be assigned within MLE the requirement to complete a competency for taking a blood sample for transfusion every three years.*

7. Specific Practice in Specialist Areas (e.g. NICU) *In specialist areas restrictions and certain exceptions apply:*

NICU: *ONLY Doctors, ANNPs, competent phlebotomists and competent nurses may undertake venepuncture.*

- Obtaining blood from a CVAD when there is a clinical need must only be performed by a competent staff, who must adhere to the Central Venous Device Access Policy for Adults (CP1g), for guidance. For management of CVAD in neonates, see local intravenous therapy policy on NICU.

- In NICU it is permitted to obtain blood using a needle and syringe. This practice may also be carried out in the Emergency Department if care is being delivered to an unwell neonate. If this is necessary a generic risk assessment must be carried out and documented in advance. Where it is appropriate, blood transfer device must be used to transfer blood from a syringe to a sampling bottle.

Hospital at Home:

- More than 2 attempts may be required when a practitioner is the lone worker with the patient, and getting help may delay the process of a diagnosis.
- When there is no printing facility at the time a decision to take a blood sample from the patient is made, the practitioner can take the blood sample but must fill in the patient's details, date and time of sampling on the blood tube, and print out the correct label and label the tube as soon as the printing facility is available.
- If the practitioner who takes the blood sample is not a registered health care professional, he/she must filled in the patient's details, date and time of sampling on the blood tube, and ask a registered health care professional to print out the correct label and label the tube as soon as the printing facility is available.

RENAL: see Appendix 1

8. Monitoring Effectiveness

The table below details the monitoring procedures which provide assurance to NBT that this Policy is being adhered to in Trust practice. It describes both the processes for monitoring compliance and the actions to be taken where shortfalls and non-compliance are identified.

What is monitored:	How?	When?	By who?	Where are the results reported and reviewed?	Where shortfalls are identified, how will improvement and learning take place?
Venepuncture related clinical practice incidences	Datix	As and when they occur	Clinical area/ward managers Line managers/supervisors Heads of Nursing, Clinical Matrons and Clinical Directors as appropriate.	Division / department governance meetings. Trust governance committees if warranted by seriousness of incident	Clinical area / ward managers are responsible for ensuring action plans are in place to support improvements in practice and interventions take place as planned. (These may include supervised practice, further training and assessment of competence)
Access to training for non-medical practitioners	Through training application	At the time of application	Clinical area/ward manager Clinical skills trainers in Staff Development	Training and competence are recorded on the MLE. Training places are allocated in conjunction with manager approval and willingness to undertake clinical area support.	Where candidates repeatedly fail to undertake completion of competency assessment, training places may be denied or restricted following discussion with clinical area managers.
Wrong blood in tube incidences	Datix	As and when they occur	Laboratory department managers	Laboratory governance meetings Trust Transfusion Committee	Clinical area / ward managers are responsible for ensuring action plans are in place to support improvements in practice and interventions take place as planned. These may include supervised practice, further training and assessment of competence)

9. Associated Documents

Overarching Infection Control Policy IC01
Sharps Management Policy HS07
Hand Hygiene Policy IC06
Standard Infection Control Precautions Policy IC05
Consent Policy for Examination and Treatment CG07
Mental Capacity Act 2005
Request Form and Specimen Labelling Policy CG45
Policy for Waste Management and the Safe Handling of Waste HS29
Patient Identification Policy CP7g
Blood Transfusion Policy CP 2a
Blood (2006)
Interim Incident Reporting Policy CG 01a
Central Venous Access Device Policy CP1g

10. References

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Available at: http://www.rmmonline.co.uk/manual/c10-sec-0036#c10-sec-0036
Accessed: 24 August 2018

Loveday HP, Wilson JA, Pratt RJ, Golsorkhi M, Tingle A, Bak A, Browne J, Prieto J, Wilcox M (2014) *Epic 3: National Evidence-Based Guidelines for Preventing Healthcare-Associated Infections in NHS Hospitals in England.* Journal of Hospital Infection 865, S1-S70. Elsevier.

National Blood Transfusion Committee (2016) *Requirements for Training and Assessment in Blood Transfusion Version1 March.*

National Blood Transfusion Committee(2016) *Appendix 1 - NBTC National Standards for the Clinical Transfusion Proces*. Standards for the Clinical Transfusion Process March.

Pages 13 – 22 not relevant to ZooTB study so have been omitted from ZooTB Research Protocol

Appendix 4

Guidelines for venepuncture procedure

Equipment needed:

Gloves and plastic apron
A 2% Chlorhexidine in 70% isopropyl alcohol swab (Or Povidine Iodine 10% if sensitive to chlorhexidine)
Safer needle device
Sample tubes
Gauze swabs
Disposable single use tourniquet
Plastic injection tray
Sharps container
Plaster (if appropriate)
Sharps box (if not using integral system as above)

NB if topical local anaesthetic is necessary this must be applied 30 to 60 minutes prior to the procedure. It must be prescribed. If an injectable local anaesthetic is to be used, the practitioner must be competent in intradermal injection technique to administer the drug.

Procedure:

1. Explain the procedure to the patient, gaining valid consent.
2. Check identity of the patient matches the details on the request form or electronically generated order labels, and verbally verified by the patient if appropriate.
3. Gather equipment together in a plastic tray with the sharps bin, and take to the bedside.
4. Assist the patient into an appropriate position to allow access to the limb for venepuncture.
5. Arrange the limb so the patient is comfortable.
6. Apply the disposable/single use tourniquet, assess and select the appropriate vein, and release the tourniquet.
7. Put on plastic apron. Wash hands using soap and water and six-step hand decontamination technique.
8. Open outer plastic packaging of the safer needle device but do not, at this point, remove the sheath of the needle. Ensure sample bottles are strategically placed to ensure they can be taken in the correct order (see order of draw, Appendix 3).
9. Decontaminate the hands and put on gloves.
10. Clean the patient's skin and the selected vein thoroughly using a 2% Chlorhexidine in 70% isopropyl alcohol wipe (or Povidine Iodine 10% if sensitive to chlorhexidine) for 30 seconds and allow to dry. Do not re-palpate the vein or touch the skin afterwards.
11. Re-apply the tourniquet.

12. Remove the sheath of the needle sheath and inspect the needle for any faults.

13. Anchor the vein by applying manual traction on the skin a few centimetres below the proposed site of insertion if required.

14. Ensure the needle is in the bevel-up position and insert the needle through the skin at the selected angle according to the depth of the vein.

15. Reduce the angle of descent of the needle as soon as the vein has been punctured if required.

16. Attach the first vacuumed sample tube by pushing it in to the vacutainer attached to the needle. Whilst doing this ensure the needle is anchored firmly to prevent through puncture of the vein. Fill the tube to the desired level.

17. Remove the first tube, (by pulling it gently back) whilst keeping the needle still; agitate the tube twice then apply any further sample tubes in accordance with the order of draw.

18. When all required samples are obtained, release the tourniquet.

19. Remove the needle, apply digital pressure directly over the puncture site (but do not apply pressure until the needle has been fully removed).

20. Activate the safety sheath to cover the needle and dispose of the needle device into the sharps container.

21. Gently invert the sample tubes as directed by the order of draw guide (the number of times varies for specific sample tubes). See Appendix 3.

22. Ask the patient where possible to state their full name and date of birth again and confirm these details match their wrist bands where used and the request form / ICE labels. Then label the tubes in the presence of the patient.

23. Before leaving the patient observe the site for signs of swelling or leakage, and ask the patient if any discomfort or pain is felt, (if appropriate).

24. Discard waste in appropriate waste bins.

25. Remove gloves and apron, and wash hands.

26. Document date, time, site and reason for venipuncture, consent and the application of aseptic non-touch technique in patient's record.

- Adapted from The Royal Marsden Manual of Clinical Nursing Procedures (9th ed)(2018)

22.2 Quantiferon Assay Protocol North Bristol NHS Trust

North Bristol NHS Trust

BLOOD SCIENCES
DEPARTMENT OF IMMUNOLOGY AND IMMUNOGENETICS

Title of Document: 4 Tube Quantiferon Assay
Q Pulse Reference N°: IMMIPSOP041 Version N°: 1.5
Authoriser: Paul Virgo

4 Tube Quantiferon Assay

1. INTRODUCTION

1.1 Purpose of the Procedure
This assay provides a test screen for TB. It is performed using a QuantiFERON®-TB Gold Plus tubes with an ELISA assay.

1.2 Staff
Qualified and trainee BMS's under supervision can perform all tests. Associate Practitioners can, after appropriate training, perform the running of the assay on the DS2 (Section 7, Method). Transmission of results can be performed by Associate Practitioner after a qualified BMS has verified the validity of the assay. The checking and emailing of results is not to be performed by the Associate Practitioner.

1.3 Related Documents
IMMAISOP021
IMMF256

2. PRINCIPLE OF THE PROCEDURE
QuantiFERON®-TB Gold Plus (QFT-Plus) has new formulations of ESAT-6/CFP-10 peptides contained in two tubes - tube 1 (TB1) and tube 2 (TB2). TB1 contains relatively long synthetic peptide cocktails to mainly stimulate CD4+ T cells, whereas TB2 also contains short peptide cocktails to stimulate both CD4+ and CD8+ T cells.
The Nil tube is used to determine background levels whilst the Mitogen tube is used as a positive control. This may also be important where there is doubt as to the individual's immune status. The Mitogen tube also serves as a control for correct blood handling and incubation.
A QFT-Plus assay is considered positive for an interferon-γ (IFN-γ) response to either TB antigen tube that is significantly above the NIL IFN-γ IU/ml value. A low response to the Mitogen (<0.5 IU/ml) indicates an indeterminate result when a blood sample also has a negative response to the TB antigens.
Detection of interferon-γ (IFN-γ) by Enzyme-Linked Immunosorbent Assay (ELISA) is used to identify *in vitro* responses to these peptide antigens that are associated with *Mycobacterium tuberculosis* infection.

QuantiFERON®-TB Gold Plus is an indirect test for *M. tuberculosis* infection (including disease) and is intended for use in conjunction with risk assessment, radiography and other medical and diagnostic evaluations.

3. SPECIMEN REQUIREMENTS
General acceptance and rejection criteria for sample quality and labeling are given in the departmental policy (TTGENSPO003)

Specimen bottles are supplied with the kit and comprise of a nil control (grey cap), TB1 antigen (green cap), TB2 antigen (yellow cap) and mitogen control (purple cap) all of which are required for the assay. Sets of bottles are stored in the Flow Cytometry section of Immunology.

A set of instructions for the assay and a request form may be required to be sent with the blood bottles (IMMF256).
Overfilled samples will not be processed for this assay.

Specimen bottles are required to be filled to the indicated line, be well mixed and returned to the laboratory within 16 hours of being taken.

4. HEALTH AND SAFETY

General laboratory safety procedures apply as outlined in the Pathology Sciences and Departmental safety codes of practice (COP001 and IMMCOP002), and procedures for handling of samples, reagents and their disposal (TTGENSOP003 and TTGENSOP006).
Quantiferon conjugate and Quantiferon IFN- γ contains Boric acid – may damage fertility or the unborn child.
Quantiferon Enzyme Stopping Solution contains Sulphuric acid – may cause severe burns and eye damage. May be corrosive to metals
Quantiferon Enzyme Substrate Solution may cause mild skin irritation.
Quantiferon Green Diluent contains trisodium 5-hydroxy-1-(4-sulphophenyl)-4-(4-sulphophenylazo) pyrazole-3-carboxylate – may cause allergic skin reaction.
Quantiferon Wash Buffer 20x concentrate contains mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-"H-isothiazol-3-one (3:1) – may cause allergic skin reaction.

5. EQUIPMENT AND INSTRUMENTATION

Dynex DS2
Hettich Rotina 46 centrifuge
VWR 37°C Incubator
Gilson P200 pipette
Gilson P1000 pipette
Gilson P5000 pipette
QuantiFERON®-TB Gold Plus tubes

All pipettes have metrological traceability

6. REAGENTS, STANDARDS, CALIBRANTS AND CONTROLS

Blood Collection Tubes
• Store tubes at 4°C to 25°C.
Use until expiry date.

Control Antigens
• Store antigens at 2°C to 8°C.

ELISA Kit Reagents
• Store kit at 2°C to 8°C.
• Always protect Enzyme Substrate Solution from direct sunlight.
Record date kit was opened on front of box.

Reconstituted and Unused Reagents
• The reconstituted Kit Standard may be kept for up to 3 months if stored at 2°C to 8°C.
° *Note the date the Kit Standard was reconstituted.*

• Once reconstituted, unused Conjugate 100X Concentrate must be returned to storage at 2°C to 8°C and must also be used within 3 months.
° *Note the date the Conjugate was reconstituted.*
• Working strength Conjugate must be used within 6 hours of preparation.
• Working strength Wash Buffer may be stored at room temperature for up to 2 weeks.

Reconstitute the freeze dried Kit Standard with the volume of deionised or distilled water indicated on the label of the Standard vial. Mix gently to minimise frothing and ensure complete solubilisation. Reconstitution of the Standard to the stated volume will produce a solution with a concentration of 8.0 IU/ml.
Reconstitute freeze dried Conjugate 100X Concentrate with 0.3ml of deionised or distilled water. Mix gently to minimise frothing and ensure complete solubilisation of the Conjugate.
Dilute one part Wash Buffer 20X Concentrate with 19 parts deionised or distilled water and mix thoroughly. Sufficient Wash Buffer 20X Concentrate has been provided to prepare 2L of Working Strength wash buffer. Record date of manufacture and expiry of diluted reagent.

Quantiferon Control Panel
The assay is controlled using the Quantiferon Control Panel kit. It consists of control samples at three levels (1, 2 and 3) within the linear range of the Quantiferon Elisa platform.
Control samples are reconstituted with 0.25ml of deionised water ensuring complete mixing. Transfer to an appropriate tube for analysis. They are processed in the same way as samples with level 1 replacing the 'Antigen 1' plasma sample, level 2 replacing the 'Antigen 2' plasma sample and level 3 replacing the 'Mitogen' plasma sample.
It is necessary to include a green diluent blank tube with the controls. Pipette 200µl of green diluent into an appropriate tube and place at the beginning of the control samples.

Lyophilized control samples are stored at or below 8°C. Reconstituted control samples must be stored at 2°C to 8°C and be used within 28 days of reconstitution.
The control samples are only used for three runs before being discarded. Record, with a mark, on the tube lid each time they are used.

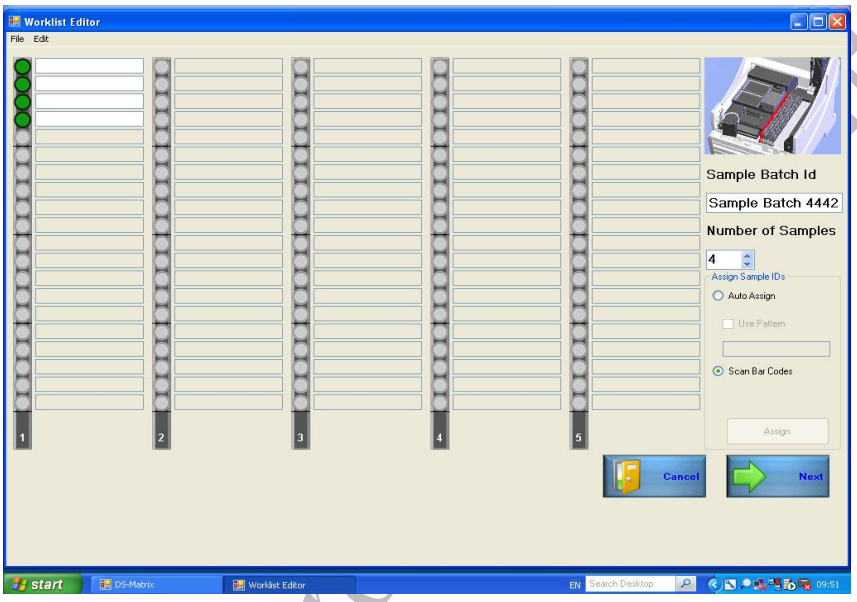
The assay requires all samples to have a Winpath barcode on them. Print barcodes from Winpath sample 17B47413 as required for use on control tubes.

Before a new batch of control samples are used in the assay it is necessary to establish the mean and SD for that batch. This is undertaken by testing five aliquots of each level at the end of a run. These results are sent to the Lead Healthcare Scientist for Immunology who will calculate the new mean and SD levels and produce new graphs and a table for data entry (see Section 8 for location details).

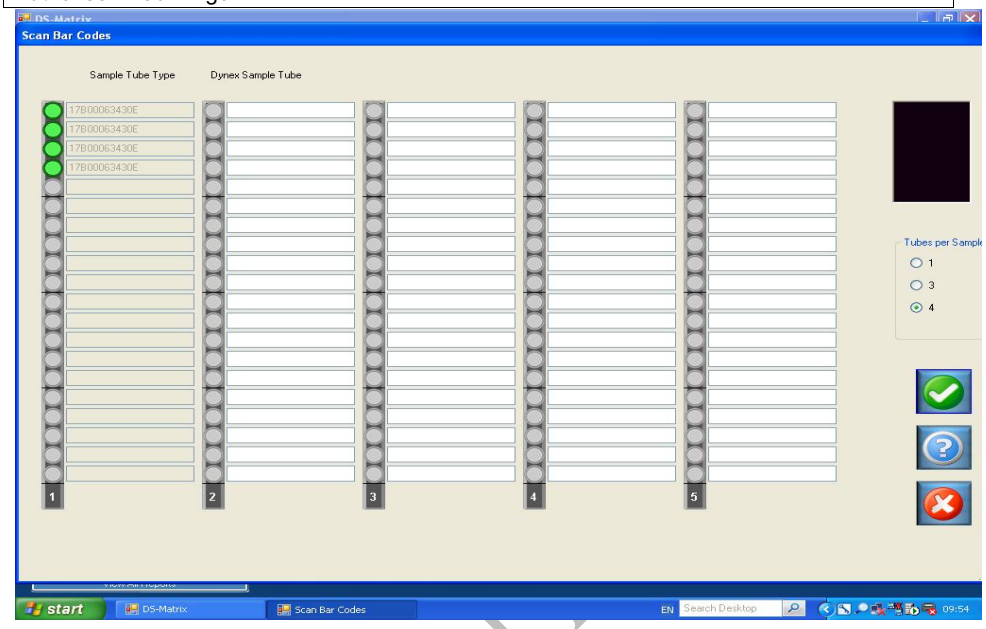
7. METHOD

1. On receipt of sample tubes invert all tubes 10 times and place in VWR 37°C Incubator at 37°C +/- 1°C for 16-24 hours.
2. After incubation centrifuge for 15 minutes at 3000 rpm.
3. Samples can be stored at 2-8°C for up to 28 days until assay is to be performed.
4. Allow samples and reagents except for conjugate concentrate to equilibrate to room temperature (22°C +/- 5°C. Allow at least 60 minutes for equilibration.
5. Switch on Dynex DS2 and start up as per IMMAISOP021.
6. From main screen select File – worklist editor.

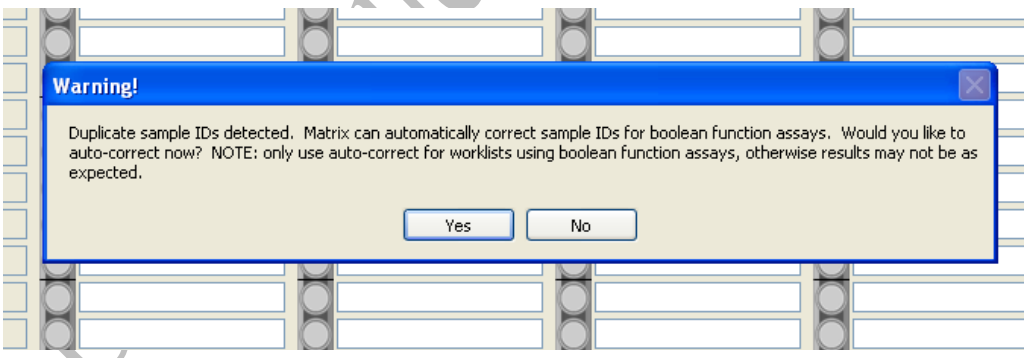
7. Ensure Use LIS on sample batch is ticked.
8. Click OK.
9. Enter number of samples in the run. For a full plate enter 88 (21 unknown samples of 4 tubes plus 4 tubes for the control sample) as 8 of the wells are required for derivation of the standard curve.
10. Ensure scan bar codes option is selected (see image below).
11. Click next.



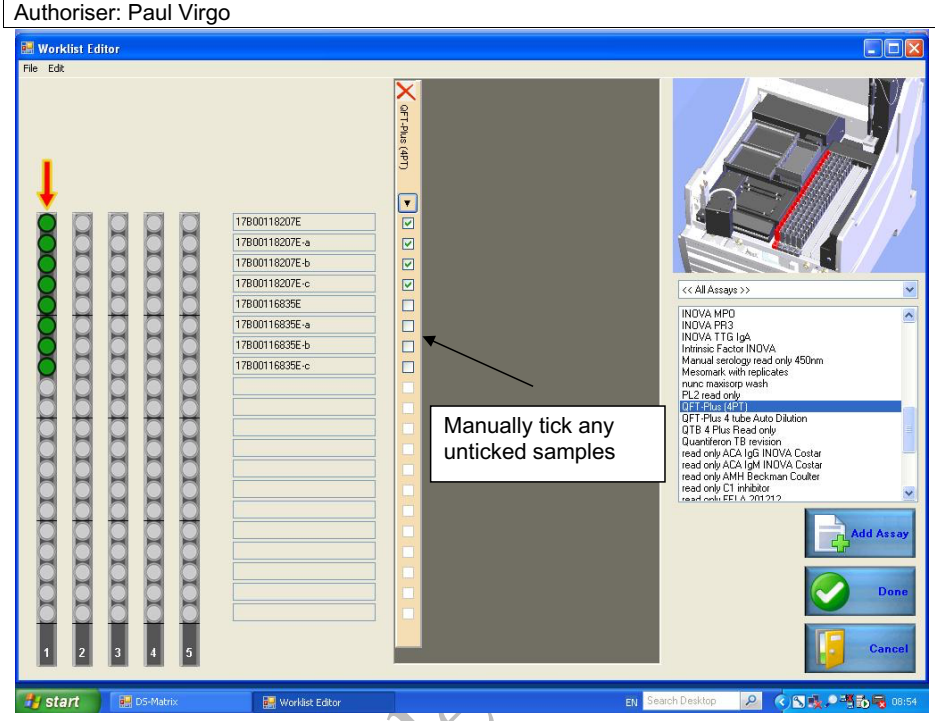
12. Click Skip on top tool bar.
13. Load samples and control tubes to sample racks. Ensure all four patient tubes are together and placed the order Neg (Grey), TB1 (green), TB2 (yellow), Mitogen (purple). All Winpath barcodes must face outwards and all must end in an E.
14. Click OK when scan bar codes box appears.
15. Slowly pull out and push in rack to allow bar codes in all racks to read.
16. Select option 4 in tubes per sample (see image below).
17. Click on green tick.



18. Click yes on Boolean function message. This allows tubes to be labelled automatically to discriminate between the four sample tubes.



19. Wait for polling LIMS message to disappear.
20. Select QFT-Plus (4PT) from assay option list (double click to apply to all tubes).
21. Ensure all boxes for each rack are ticked. It is necessary to manually tick each box for the control samples (see image below).

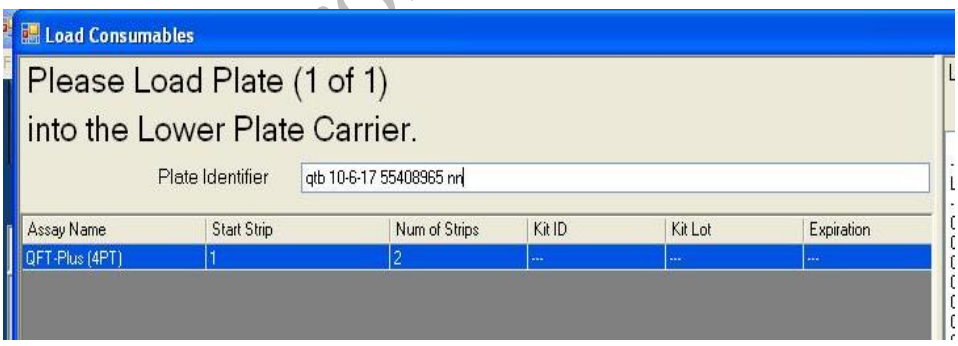


22. Click done.
23. Wait for plate message to disappear.
24. Click Accept then click Skip.
25. Load consumables as indicated. Use the correct volumes and ensure they are placed in the correct position. Click on green tick when each consumable is loaded.
26. Working strength conjugate is prepared by putting the required amount of conjugate concentrate in Green Diluent as below. Mix thoroughly but avoid frothing.

NUMBER OF STRIPS	VOLUME OF CONJUGATE 100X CONCENTRATE	VOLUME OF GREEN DILUENT
2	10µL	1.0mL
3	15µL	1.5mL
4	20µL	2.0mL
5	25µL	2.5mL
6	30µL	3.0mL
7	35µL	3.5mL
8	40µL	4.0mL
9	45µL	4.5mL
10	50µL	5.0mL
11	55µL	5.5mL
12	60µL	6.0mL

27. Use reconstituted Kit Standard to produce a 1 in 4 dilution series of IFN-γ in Green Diluent (GD) as below.
    a. Label 4 tubes S1, S2, S3, S4.
    b. Add 300µl of GD to S1, S2, S3, S4.
    c. Add 300µl of Kit Standard to S1 and mix thoroughly.
    d. Transfer 100µl from S1 to S2 and mix thoroughly.
    e. Transfer 100µl from S2 to S3 and mix thoroughly.
    f. GD alone serves as the zero standard (S4).

28. Add plate. Check the number of strips required and adjust accordingly. Label plate in identifier with qtb, date, kit lot number and your initials (see example below). Click green tick when done.



29. Check all tip boxes are filled.
30. Add sufficient wash buffer to the correct reservoir.
31. Check clean fluid is sufficient.
32. Ensure waste containers are empty.
33. Click Skip.

34. Allow analyser to run. If sample cannot be added by analyser, select option to add sample manually and follow on screen instructions. Ensure sample is added to the correct well.
35. After assay is complete remove all reagents and samples, empty waste containers and reset consumables from drop down menu on top tool bar.
36. Select assay name from list and print results.

8. QUALITY CONTROL

The accuracy of test results is dependent on the generation of an accurate standard curve. Therefore, results derived from the standards must be examined before test sample results can be interpreted.
For the ELISA to be valid:
• The mean OD value for Standard 1 must be ≥ 0.600.
• The %CV for Standard 1 and Standard 2 replicate OD values must be ≤15%.
• Replicate OD values for Standard 3 and Standard 4 must not vary by more than 0.040 optical density units from their mean.
• The correlation coefficient (r) calculated from the mean absorbance values of the standards must be ≥ 0.98.

The QFT-Plus Analysis Software calculates and reports these quality control parameters. If the above criteria are not met the run is invalid and must be repeated.

• The mean OD value for the Zero Standard (Green Diluent) should be ≤ 0.150.
If the mean OD value is > 0.150 the plate washing procedure should be investigated.

Control samples results are plotted on Shewart charts, these are located on the L Drive/ImmunologyImmunogenetics/Batch acceptance&IQC/QTB QC). The values to be plotted are taken from the data column.
Control results which are out of the indicated range must be shown to the Lab Manager or Lead Health Care Scientist for assay validation before reporting can proceed. It is possible that the assay is rejected and must be repeated.
Control samples that are repeatedly out of range should be brought to the attention of the Flow Section management team and Lead Healthcare Scientist for review of the calculated control means and SDs.

9. LIMITATIONS OF THE EXAMINATION

Individuals with Nil values greater than 8 IU/mL are classed as “indeterminate” because a 25% higher response to ESAT-6 and/or CFP-10 antigens may be outside the assay measurement range.
Unreliable or indeterminate results may occur due to:
• Deviations from the procedure described in the package insert,
• Excessive levels of circulating IFN-γ or presence of heterophile antibodies,
• Longer than 16 hours from blood specimen drawing to incubation at 37°C,
• Insufficient mixing at blood taking stage,
• Lymphocyte count is too low,
• Patient is immunosuppressed.

A negative QFT result does not preclude the possibility of *M. tuberculosis* infection or tuberculosis disease: false negative results can be due to stage of infection (e.g., specimen

obtained prior to the development of cellular immune response), co-morbid conditions which affect immune functions, incorrect handling of the blood collection tubes following venipuncture, incorrect performance of the assay, or other immunological variables.

A positive QFT result should not be the sole or definitive basis for determining infection with *M. tuberculosis*. Incorrect performance of the assay may cause false-positive responses.

A positive QFT result should be followed by further medical evaluation and diagnostic evaluation for active tuberculosis disease (e.g., AFB smear and culture, chest X-ray).

While ESAT-6, CFP-10, and TB7.7(p4) are absent from all BCG strains and from most known non tuberculous mycobacteria, it is possible that a positive QFT result may be due to infection by *M. kansasii*, *M. szulgai*, or *M. marinum*. If such infections are suspected, alternative tests should be investigated.

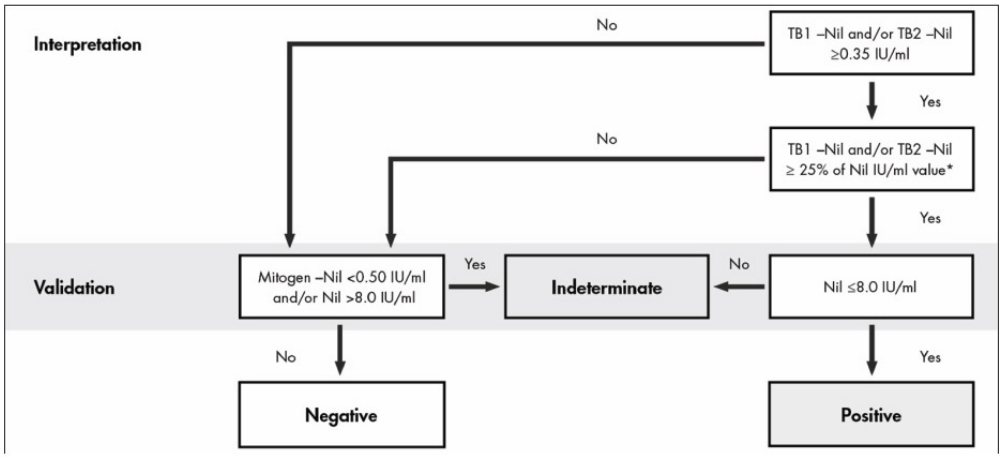
10. RECORDING AND CALCULATION OF RESULTS

Results are calculated by the analyser software. It is worth just checking all results to look for possible sample transposition or any abnormal findings. These samples can be retested on the next run.
In the event that the software cannot calculate results then follow the manual procedure below.
Important note: Diagnosing or excluding TB and assessing the probability of LTBI, requires a combination of epidemiological, historical, medical, and diagnostic findings that should be taken into account when interpreting QFT-Plus results.

Use flow chart and/or table below to determine result by calculating the required values from the measured results. Write result on worksheet and initial and date when satisfied results are correct. Check these results are the same as the analyser interpreted results.

Nil (IU/ml)	TB1 minus Nil (IU/ml)	TB2 minus Nil (IU/ml)	Mitogen minus Nil (IU/ml)*	QFT-Plus Result	Report/Interpretation
≤8.0	≥0.35 and ≥ 25% of Nil value	Any	Any	Positive†	*M. tuberculosis* infection likely
	Any	≥0.35 and ≥ 25% of Nil value			
	<0.35 or ≥0.35 and <25% of Nil value	<0.35 or ≥0.35 and <25% of Nil value	≥0.5	Negative	*M. tuberculosis* infection NOT likely
	<0.35 or ≥0.35 and <25% of Nil value	<0.35 or ≥0.35 and <25% of Nil value	<0.5	Indeterminate‡	Likelihood of *M. tuberculosis* infection cannot be determined
>8.0§	Any				

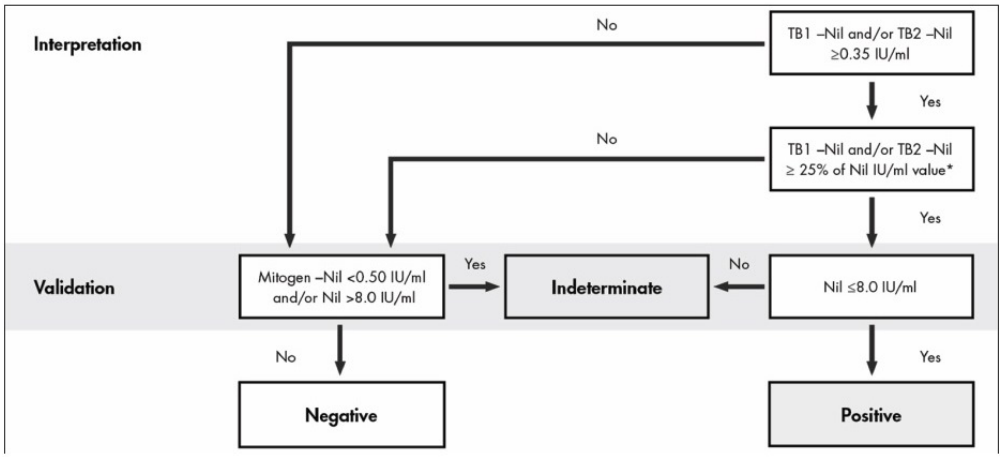
∗ Responses to the Mitogen positive control (and occasionally TB Antigens) can be outside the range of the microplate reader. This has no impact on test results. Values >10 ml are reported by the QFT-Plus software as >10 IU/ml.



11. BIOLOGICAL REFERENCE VALUES
N/A.

Nil (IU/ml)	TB1 minus Nil (IU/ml)	TB2 minus Nil (IU/ml)	Mitogen minus Nil (IU/ml)*	QFT-Plus Result	Report/Interpretation
≤8.0	≥0.35 and ≥ 25% of Nil value	Any	Any	Positive†	*M. tuberculosis* infection likely
	Any	≥0.35 and ≥ 25% of Nil value			
	<0.35 or ≥0.35 and <25% of Nil value	<0.35 or ≥0.35 and <25% of Nil value	≥0.5	Negative	*M. tuberculosis* infection NOT likely
	<0.35 or ≥0.35 and <25% of Nil value	<0.35 or ≥0.35 and <25% of Nil value	<0.5	Indeterminate‡	Likelihood of *M. tuberculosis* infection cannot be determined
>8.0§	Any				

∗ Responses to the Mitogen positive control (and occasionally TB Antigens) can be outside the range of the microplate reader. This has no impact on test results. Values >10 ml are reported by the QFT-Plus software as >10 IU/ml.



11. BIOLOGICAL REFERENCE VALUES
N/A.

12. MEASUREMENT UNCERTAINLY OF MEASURED QUANTITY VALUES

Current estimates of measurement uncertainty are documented in IMMQVAL02.

13. REPORTING AND VALIDATION OF RESULTS

Once all QC checks and control sample checks have passed the results can be transmitted from the analyser to Winpath.

An easy way to check that results have been transmitted is to pull up outstanding QTB (Winpath test set for Quantiferon) test list in Winpath before result transmission and then check that the number outstanding after transmission has reduced by the number of expected results.

Method to transmit results on DS2.

1. Select LIS-Link from desktop.
2. Enter password – dynex.
3. Click Ok.
4. Open up assays to see number of quantiferon results transmissible.
5. Click on Quan.
6. From operations option in toolbar select Commit operations then Send data to LIS host.
7. Wait for LIS-Link box to state finished transmitting results to LIS host.
8. Click Ok.
9. Wait for returning results to LIS host box to disappear.
10. Results have now been transmitted so LIS-link can be closed.

Any results needed to be entered manually are recorded as either positive or negative or indeterminate in Winpath in the test QTB. Enter positive, negative or the comment @INDE for indeterminate.

Positive and indeterminate results for patients from Occupational Health at NBT, UBH (APOCC) and Weston General need to be emailed to occupationalhealth@uhbristol.nhs.uk
Positive and indeterminate results for paediatric patients from Bristol Childrens Hospital should be emailed to Jolanta.Bernatoniene@UHBristol.nhs.uk, Marion.Roderick@UHBristol.nhs.uk, Stefania.Vergnano@UHBristol.nhs.uk, Florence.Manyika@UHBristol.nhs.uk and Alice.Parham@UHBristol.nhs.uk

14. REFERENCES

Refer to kit insert for more information or visit www.QuantiFERON.com

22.3 Participant information and invite sheet

Estimating the burden of zoonotic tuberculosis in Southwest England: The ZooTB Study
Study Information and Invitation

Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, BS8 2BN
zootb-study@bristol.ac.uk; 07553 785198

About the study
We are investigating whether people who work with cattle infected with bovine TB develop an immune response to bovine TB themselves. We are asking around 160 people who have been in contact with TB-infected cattle to take a blood test, this will determine if you have a latent TB infection. We will also ask about your interaction with cattle and milk products, allowing us to investigate how bovine TB may be spread from cattle to humans. This research follows on from a pilot study we conducted in October 2021 at The Dairy Show.

Why is this research important?
Each year in the UK, 30 to 40 people are diagnosed with TB due to *M. bovis* – the main cause of TB in cattle. *M. bovis* disease in people is known as zoonotic tuberculosis, which is why the study is called ZooTB. The symptoms of zoonotic TB are similar to other TB symptoms in people: a persistent cough, weight loss, fever, loss of appetite. We do not have an accurate estimate for the number of people who might have been exposed to *M. bovis* as a result of their occupational risk i.e., working with TB infected cattle - this study will help answer this.

Who can take part?
Anyone aged over 18 years and who has worked with confirmed TB-infected cattle located in Southwest England can take part.

What will happen if I take part?
Taking part involves:

1) ***Completing the consent form online (****accessed via the link at the end of this document)*
2) ***Completing a short questionnaire about you and your contact with cattle:*** The questionnaire can be completed in advance online or at the study site. This will take about 15 minutes to complete.
3) ***Providing a blood sample:*** You will have a blood sample taken by an experienced phlebotomist at a study site.

As a thank you for taking part we will provide you with either a £5 food and drink voucher or equivalent light lunch/supper at the event.

Where are the study sites?
We will host study test days at agricultural shows and events in Southwest England. Details of upcoming study test days will be listed on the study website: www.bristol.ac.uk/zootb

What do I do next if I want to take part?
Additional study details are listed on the next page of this information sheet. *Please read these carefully.*
If you would like to take part in this study, click on the link (or copy and paste/type into your web browser)
https://redcap.link/zootb

Participation in the study is completely voluntary and there is no obligation to take part. If you have any questions, please get in touch with us directly at the study site or using the contact details at the top of this letter.

Thank you for finding out about the ZooTB study.

Drs Amy Thomas and Ellen Brooks-Pollock (study investigators)

University of BRISTOL NHS North Bristol NHS Trust ZOO TB NHS University Hospitals Bristol and Weston NHS Foundation Trust

Estimating the burden of zoonotic tuberculosis in Southwest England: The ZooTB Study
Study Information and Invitation

Should I take part if I have no symptoms?
Yes, please! People with latent TB infection don't have any symptoms. Most people (90%) will never go on to develop symptoms. We are using the latent TB test to determine the risk of exposure to *M. bovis* in farmers and people who work with cattle. This will be important for the 10% who may go on to get symptoms.

How will my blood sample be used?
After collection, your blood sample will be transported to North Bristol NHS Trust laboratory, where it will be tested for evidence of latent TB using the Interferon Gamma Release Assay (IGRA). This test assesses if you have been previously infected with *Mycobacterium sp*. by measuring an immune response to the bacterium. The test will be started as soon as the laboratory receive the sample. No information that could identify you would be given to anyone using the samples. Once the test has been completed, usually within 3 days after sample collection, your blood sample will be destroyed. However, you can consent for an optional extra blood sample to be collected and donated to the Bristol Biobank (https://directory.biobankinguk.org/Profile/Biobank/GBR-1-112), so that it can be used for further research relating to infection and immunity.

What laboratory test will we conduct with your blood sample?
One method to assess if someone has been previously infected with *Mycobacterium sp.* is to measure a person's immune response to the bacterium using a blood-based test, called the Interferon Gamma Release Assay (IGRA).

What will happen if I get a positive test?
We will inform you and your GP if your IGRA blood test is positive. A positive IGRA means that you likely have latent TB, caused by previous infection with either the bovine TB bacterium (*M. bovis*) or the human TB bacterium (*M. tuberculosis*). The majority of individuals with latent TB never develop any problems, but a small proportion may develop symptoms and benefit from treatment. Even if you do not develop symptoms, you might want to discuss this further. We would recommend contacting your GP, who can refer you to the local TB service if required.

What will happen if I get an indeterminate or negative test?
We will inform you and your GP if your IGRA blood test is indeterminate. An indeterminate test means that we can't conclude that your blood sample is negative or positive. Even if you do not develop symptoms, you might want to discuss this further. We will inform only you if your test is negative. A negative test result indicates that *Mycobacterium* infection is unlikely.

What are the possible benefits if I take part in the study?
By taking part you will be directly contributing to advancing our knowledge of zoonotic TB in the UK farming community. This will help improve policy and measures to support bovine TB and zoonotic TB control.

How will we use information about you?
We will need to use information from you for this research project. This information will include your: initials, name, date of birth, contact details and GP contact details. People will use this information to do the research or to check your records to make sure that the research is being done properly. People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead. We will keep all information about you safe and secure. Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

Estimating the burden of zoonotic tuberculosis in Southwest England: The ZooTB Study
Study Information and Invitation

What are your choices about how your information is used?
You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have. We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you. If you agree to take part in this study, you will have the option to take part in future research using your data saved from this study (data will be transferred to the University of Bristol Research Data Storage Facility [RDSF]. The RDSF provides secure, RCUK compliant long-term storage for research data).

Where can you find out more about how your information is used?
You can find out more about how we use your information by contacting the research team (contact details are on page 1) or the University of Bristol Data Protection Officer (data-protection@bristol.ac.uk).

Can I withdraw from the study?
You can stop being part of the study at any time, without giving a reason.

Who is responsible for this research?
Scientists are conducting this research under the responsibility of the University of Bristol. The study has been approved by the NHS Research Ethics Committee (Ref: 21/YH/0241) and it is funded by The Wellcome Trust.

What if something goes wrong or I wish to complain?
If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact the study team (contact details on page 1). If you have any further complaints or concerns, you can contact the Research Governance Team, University of Bristol, Trinity Street, One Cathedral Square, Bristol, BS5 5DD (research-governance@bristol.ac.uk). In the very unlikely event that you are injured there is no automatic insurance protection to compensate you, but you can still make a legal claim, the University has Public Liability Insurance that covers its legal liability in relation to study participation.

Thank you for taking the time to read and consider this information.

22.4 Positive IGRA result letter and accompanying information sheet

IGRA Results and Information: The ZooTB Study

Bristol Veterinary School, University of Bristol, Churchill Building, Langford, Bristol, BS40 5DU
zootb-study@bristol.ac.uk; 07553 785198

Dear xxxxx,

Thank you for taking part in the ZooTB study, your involvement is allowing us to investigate the critical link between infection of *M. bovis* in cattle and the risk this might pose to people who work with cattle. We are currently analysing questionnaire responses alongside blood-based interferon gamma release assay (IGRA) results. A summary of our study findings will be available at the end of the study and updates will be provided on the study website www.bristol.ac.uk/zootb

Following analysis of your blood sample, your IGRA result is **POSITIVE**.
A positive IGRA blood test means that you likely have latent TB, caused by infection with either the bovine TB bacterium (*M. bovis*) or potentially the human TB bacterium (*M. tuberculosis*). The vast majority of individuals with latent TB never develop any problems, but a small proportion develop symptoms and need antibiotic treatment.

Even if you do not develop symptoms, you might want to discuss this result further. We have informed your GP of your result and recommend contacting them for further discussion. They will be able to refer you to the local TB service if this is required, and can also let you know what symptoms to look out for.

Please see the enclosed information sheet for a re-cap on the IGRA, information on interpreting this test result and possible next steps. If you have any queries relating to your result or any aspect of the ZooTB study please contact a member of the study team via email (zootb-study@bristol.ac.uk) or telephone (07553 785198)

Thank you again for your participation in the ZooTB Study.

Yours sincerely,

Dr Amy Thomas

Principal Investigator

Dr Ellen Brooks-Pollock

Chief Investigator

Understanding your IGRA result: The ZooTB Study

Bristol Veterinary School, University of Bristol, Langford, Bristol, BS40 5DU
zootb-study@bristol.ac.uk; 07553 785198

What is the interferon gamma release assay?

The Interferon Gamma Release Assay is a laboratory blood-based test used to determine if a person has a measurable immune response to *Mycobacterium bovis* or *Mycobacterium tuberculosis.* It is used as a test for latent TB. The test measures production of interferon gamma (IFN-γ), a component of the immune system. In the test, IFN-γ is released by certain immune cells in the blood when the are mixed with components of *Mycobacterium sp.* The immune cells will only release IFN-γ if they have previously seen *Mycobacterium*.

My result is positive, what does this mean?

A positive result indicates previous infection with *Mycobacterium sp.* is likely.
Latent TB infection is not infectious and does not make you unwell, but over a lifetime there is a low risk of it reactivating. Because of your occupational exposure to TB infected cattle, we assume that this infection was with *M. bovis*. However, the IGRA can't differentiate between *M. tuberculosis*, which only infects humans, and *M. bovis*, which infects cattle and other animals.

Will I know when I was infected?

No, we won't be able to know when you may have first been infected with *M. bovis,* this is because the IGRA measures an immune response to a past infection. The infection will either have been cleared by your immune system at some time in the past or have become 'latent' (asleep).

What should I look out for?

Latent TB is nothing to worry about and you are not infectious to others. A large number of people have latent TB infection and most never have any problems. However, certain people benefit from treatment to eradicate it and reduce the chance of TB reactivation in later life. So that you and others who may have been in close proximity to TB-infected animals are aware of the possible symptoms of tuberculosis, these may be any of the following:

- Persistent cough (more than three weeks)
- Coughing up blood at any time
- Fever
- Night sweats
- Unexplained weight loss
- Loss of appetite
- Swelling of one or more glands in the neck
- Extreme fatigue and tiredness

If you have any immediate concerns about your health, or that of your family or colleagues, then you should consult your GP in the usual way.

Do I need to do anything?

If you provided us with your GP details we'll let them know your result, if you didn't, we advise for you to register with your local GP and show them your result letter, this information sheet, and the Participant Information Sheet. We suggest that your GP refers you to a local hospital for further non-urgent discussion.

If you have any queries relating to the ZooTB study please contact a member of the study team via email (zootb-study@bristol.ac.uk) or telephone (07553 785198).

22.5 Negative IGRA result letter and accompanying information sheet

IGRA Results and Information: The ZooTB Study

Bristol Veterinary School, University of Bristol, Churchill Building, Langford, Bristol, BS40 5DU
zootb-study@bristol.ac.uk; 07553 785198

Dear xxxxx,

Thank you for taking part in the ZooTB study, your involvement is allowing us to investigate the critical link between infection of *M. bovis* in cattle and the risk this might pose to people who work with cattle. We are currently analysing questionnaire responses alongside blood-based interferon gamma release assay (IGRA) results. A summary of our study findings will be available at the end of the study and updates will be provided on the study website www.bristol.ac.uk/zootb

Following analysis of your blood sample, your IGRA result is **NEGATIVE**.

Please see the enclosed information sheet for a re-cap on the IGRA and information on interpreting this result. If you have any queries relating to your result or any aspect of the ZooTB study please contact a member of the study team via email (zootb-study@bristol.ac.uk) or telephone (07553 785198)

Thank you again for your participation in the ZooTB Study.

Yours sincerely,

Dr Amy Thomas

Principal Investigator

Dr Ellen Brooks-Pollock

Chief Investigator

Understanding your IGRA result: The ZooTB Study

Bristol Veterinary School, University of Bristol, Langford, Bristol, BS40 5DU
zootb-study@bristol.ac.uk; 07553 785198

What is the interferon gamma release assay?

The Interferon Gamma Release Assay is a laboratory blood-based test used to determine if a person has a measurable immune response to *Mycobacterium bovis* or *Mycobacterium tuberculosis.* It is used as a test for latent TB. The test measures production of interferon gamma (IFN-γ), a component of the immune system. In the test, IFN-γ is released by certain immune cells in the blood when the are mixed with components of *Mycobacterium sp.* The immune cells will only release IFN-γ if they have previously seen *Mycobacterium*.

My result is negative, what does this mean?

A negative test result indicates that *Mycobacterium* infection is unlikely.

If you have any queries relating to the ZooTB study please contact a member of the study team via email (zootb-study@bristol.ac.uk) or telephone (07553 785198)

Thank you for taking part in the ZooTB study, you can keep up to date with study progress online
www.bristol.ac.uk/zootb

22.6 *Indeterminate IGRA result letter and accompanying information sheet*

IGRA Results and Information: The ZooTB Study

Bristol Veterinary School, University of Bristol, Churchill Building, Langford, Bristol, BS40 5DU
zootb-study@bristol.ac.uk; 07553 785198

Dear xxxxx,

Thank you for taking part in the ZooTB study, your involvement is allowing us to investigate the critical link between infection of *M. bovis* in cattle and the risk this might pose to people who work with cattle. We are currently analysing questionnaire responses alongside blood-based interferon gamma release assay (IGRA) results. A summary of our study findings will be available at the end of the study and updates will be provided on the study website www.bristol.ac.uk/zootb

Following analysis of your blood sample, your IGRA result is **INDETERMINATE**.
We cannot conclude if your test is positive or negative on this occasion.

Even if you do not develop symptoms, you might want to discuss this result further. We have informed your GP of your result and recommend contacting them for further discussion.
We advise that you are familiar of possible symptoms of tuberculosis (given on the enclosed information sheet) so that in the unlikely event these arise, you can contact your GP for further consultation and referral to the local hospital.

Please see the enclosed information sheet for a re-cap on the IGRA and information on interpreting this result. If you have any queries relating to your result or any aspect of the ZooTB study please contact a member of the study team via email (zootb-study@bristol.ac.uk) or telephone (07553 785198)

Thank you again for your participation in the ZooTB Study.

Yours sincerely,

Dr Amy Thomas

Principal Investigator

Dr Ellen Brooks-Pollock

Chief Investigator

Understanding your IGRA result: The ZooTB Study

Bristol Veterinary School, University of Bristol, Langford, Bristol, BS40 5DU
zootb-study@bristol.ac.uk; 07553 785198

What is the interferon gamma release assay?

The Interferon Gamma Release Assay is a laboratory blood-based test used to determine if a person has a measurable immune response to *Mycobacterium bovis* or *Mycobacterium tuberculosis.* It is used as a test for latent TB. The test measures production of interferon gamma (IFN-γ), a component of the immune system. In the test, IFN-γ is released by certain immune cells in the blood when the are mixed with components of *Mycobacterium sp.* The immune cells will only release IFN-γ if they have previously seen *Mycobacterium*.

My result is indeterminate, what happens next?

An indeterminate test means that we can't conclude that your blood sample is negative or positive. This can happen occassionally, for example, there could be high background levels of IFN-γ during the test. We won't contact you for a further blood sample to repeat the test. We will take this result as final and we'll let your GP know your result.

What should I look out for?

If your result had been negative, this would indicate that *Mycobacterium* infection is unlikely.

If your result had been positive, latent TB is nothing to worry about, and you are not infectious to others. A large number of people have latent TB infection and most never have any problems. However, certain people benefit from treatment to eradicate it and reduce the chance of TB reactivation in later life. So that you and others who may have been in close proximity to TB-infected animals are aware of the possible symptoms of tuberculosis, these may be any of the following:

- Persistent cough (more than three weeks)
- Coughing up blood at any time
- Fever
- Night sweats
- Unexplained weight loss
- Loss of appetite
- Swelling of one or more glands in the neck
- Extreme fatigue and tiredness

If you have any immediate concerns about your health, or that of your family or colleagues, then you should consult your GP in the usual way.

If you have any queries relating to the ZooTB study please contact a member of the study team via email (zootb-study@bristol.ac.uk) or telephone (07553 785198)

Thank you for taking part in the ZooTB study, you can keep up to date with study progress online www.bristol.ac.uk/zootb

22.7 Indeterminate IGRA result letter and accompanying information sheet for GP

IGRA Results and Information: The ZooTB Study

Bristol Veterinary School, University of Bristol, Churchill Building, Langford, Bristol, BS40 5DU
zootb-study@bristol.ac.uk; 07553 785198

Dear Doctor,

We are writing to inform you that a patient registered with you has taken part in the ZooTB study (www.bristol.ac.uk/zootb). This letter is for your information.
The ZooTB study aims to investigate the prevalence and risk factors for zoonotic TB infection in individuals occupationally exposed to cattle with bovine TB. Participation involves completing a short questionnaire and providing a blood sample for latent TB testing, conducted by collaborators at North Bristol NHS Trust using the Interferon Gamma Release Assay (IGRA).

[Patient name; DoB; first line address] has tested indeterminate, neither concluding that previous infection with *Mycobacterium sp.* is likely (positive result) or unlikely (negative result). Indeterminate results can arise in patients who are immunosuppressed.

We have notified the participant of their result and supplied them with the enclosed information sheet.
We will not be conducting any further confirmatory tests – we will take this indeterminate result as final.
We appreciate that the appropriate action following the result of a test that would not normally be indicated is not obvious. We advise that you and the participant are familiar of possible symptoms of tuberculosis (given on the enclosed information sheet) so that in the unlikely event these arise, the participant knows to re-contact you for further consultation and referral to their local hospital for non-urgent discussion.
If you are uncertain, you can discuss the participants result further with Dr Ed Moran (Consultant in Infectious Diseases) at Southmead Hospital, Bristol (Ed.Moran@nbt.nhs.uk; Tel: 0117 414 3420)

If you have any queries relating to the ZooTB study please contact a member of the study team via email (zootb-study@bristol.ac.uk) or telephone (07553 785198)

Yours sincerely,

Dr Amy Thomas	Dr Ellen Brooks-Pollock
Principal Investigator	Chief Investigator

University of BRISTOL NHS North Bristol NHS Trust ZOO TB NHS University Hospitals Bristol and Weston NHS Foundation Trust

Understanding your IGRA result: The ZooTB Study

Bristol Veterinary School, University of Bristol, Langford, Bristol, BS40 5DU
zootb-study@bristol.ac.uk; 07553 785198

What is the interferon gamma release assay?

The Interferon Gamma Release Assay is a laboratory blood-based test used to determine if a person has a measurable immune response to *Mycobacterium bovis* or *Mycobacterium tuberculosis.* It is used as a test for latent TB. The test measures production of interferon gamma (IFN-γ), a component of the immune system. In the test, IFN-γ is released by certain immune cells in the blood when the are mixed with components of *Mycobacterium sp.* The immune cells will only release IFN-γ if they have previously seen *Mycobacterium*.

My result is indeterminate, what happens next?

An indeterminate test means that we can't conclude that your blood sample is negative or positive. This can happen occassionally, for example, there could be high background levels of IFN-γ during the test. We won't contact you for a further blood sample to repeat the test. We will take this result as final and we'll let your GP know your result.

What should I look out for?

If your result had been negative, this would indicate that *Mycobacterium* infection is unlikely.

If your result had been positive, latent TB is nothing to worry about, and you are not infectious to others. A large number of people have latent TB infection and most never have any problems. However, certain people benefit from treatment to eradicate it and reduce the chance of TB reactivation in later life. So that you and others who may have been in close proximity to TB-infected animals are aware of the possible symptoms of tuberculosis, these may be any of the following:

- Persistent cough (more than three weeks)
- Coughing up blood at any time
- Fever
- Night sweats
- Unexplained weight loss
- Loss of appetite
- Swelling of one or more glands in the neck
- Extreme fatigue and tiredness

If you have any immediate concerns about your health, or that of your family or colleagues, then you should consult your GP in the usual way.

If you have any queries relating to the ZooTB study please contact a member of the study team via email (zootb-study@bristol.ac.uk) or telephone (07553 785198)

Thank you for taking part in the ZooTB study, you can keep up to date with study progress online www.bristol.ac.uk/zootb

22.8 Positive IGRA result letter and accompanying information sheet for GP

IGRA Results and Information: The ZooTB Study

Bristol Veterinary School, University of Bristol, Churchill Building, Langford, Bristol, BS40 5DU
zootb-study@bristol.ac.uk; 07553 785198

Dear Doctor,

We are writing to inform you that a patient registered with you has taken part in the ZooTB study (www.bristol.ac.uk/zootb). This letter is for your information.
The ZooTB study aims to investigate the prevalence and risk factors for zoonotic TB infection in individuals occupationally exposed to cattle with bovine TB. Participation involves completing a short questionnaire and providing a blood sample for latent TB testing, conducted by collaborators at North Bristol NHS Trust using the Interferon Gamma Release Assay (IGRA).

[Patient name; DoB; first line address] has tested positive, indicating previous infection with *Mycobacterium sp.* is likely. We assume IGRA positivity to be attributable to previous infection with *Mycobacterium bovis* given the participants occupational exposure. They do not have symptoms of TB, and the infection will either have been cleared by their immune system at some time in the past or have become 'latent' (asleep). Latent TB infection is not infectious and does not make you unwell but over a lifetime there is a low risk of it reactivating.

We have notified the participant of their result and supplied them with the enclosed information sheet.
We appreciate that the appropriate action following the result of a test that would not normally be indicated is not obvious. We suggest you refer them to their local hospital for further non-urgent discussion.
If you are uncertain, you can discuss the participants result further with Dr Ed Moran (Consultant in Infectious Diseases) at Southmead Hospital, Bristol (Ed.Moran@nbt.nhs.uk; Tel: 0117 414 3420)

If you have any queries relating to the ZooTB study please contact a member of the study team via email (zootb-study@bristol.ac.uk) or telephone (07553 785198)

Yours sincerely,

Dr Amy Thomas	Dr Ellen Brooks-Pollock
Principal Investigator	Chief Investigator

Understanding your IGRA result: The ZooTB Study

Bristol Veterinary School, University of Bristol, Langford, Bristol, BS40 5DU
zootb-study@bristol.ac.uk; 07553 785198

What is the interferon gamma release assay?

The Interferon Gamma Release Assay is a laboratory blood-based test used to determine if a person has a measurable immune response to *Mycobacterium bovis* or *Mycobacterium tuberculosis.* It is used as a test for latent TB. The test measures production of interferon gamma (IFN-γ), a component of the immune system. In the test, IFN-γ is released by certain immune cells in the blood when the are mixed with components of *Mycobacterium sp.* The immune cells will only release IFN-γ if they have previously seen *Mycobacterium*.

My result is positive, what does this mean?

A positive result indicates previous infection with *Mycobacterium sp.* is likely.
Latent TB infection is not infectious and does not make you unwell, but over a lifetime there is a low risk of it reactivating. Because of your occupational exposure to TB infected cattle, we assume that this infection was with *M. bovis*. However, the IGRA can't differentiate between *M. tuberculosis*, which commonly infects humans, and *M. bovis*, which commonly infects cattle and other animals.

Will I know when I was infected?

No, we won't be able to know when you may have first been infected with *M. bovis,* this is because the IGRA measures an immune response to a past infection. The infection will either have been cleared by your immune system at some time in the past or have become 'latent' (asleep).

What should I look out for?

Latent TB is nothing to worry about and you are not infectious to others. A large number of people have latent TB infection and most never have any problems. However, certain people benefit from treatment to eradicate it and reduce the chance of TB reactivation in later life. So that you and others who may have been in close proximity to TB-infected animals are aware of the possible symptoms of tuberculosis, these may be any of the following:

- Persistent cough (more than three weeks)
- Coughing up blood at any time
- Fever
- Night sweats
- Unexplained weight loss
- Loss of appetite
- Swelling of one or more glands in the neck
- Extreme fatigue and tiredness

If you have any immediate concerns about your health, or that of your family or colleagues, then you should consult your GP in the usual way.

Do I need to do anything?

If you provided us with your GP details we'll let them know your result, if you didn't, we advise for you to register with your local GP and show them your result letter, this information sheet, and the Participant Information Sheet. We suggest that your GP refers you to a local hospital for further non-urgent discussion.

If you have any queries relating to the ZooTB study please contact a member of the study team via email (zootb-study@bristol.ac.uk) or telephone (07553 785198).

22.9 *Pre-screening questionnaire and consent script*

ZooTB Study: Estimating the burden of zoonotic tuberculosis in Southwest England
Script for pre-screening questions and consent hosted on REDCap

Consent will be collected on REDCap.
Pre-screening questions will capture if an individual meets the inclusion criteria and can proceed to collect consent.

[Script for welcome page]

Thank you for your interest in taking part in the ZooTB study.
Before we proceed to collecting your consent to take part, we need to ask you a few questions to check your eligibility.

Pre-screening questions

Do you have active involvement with cattle who have had confirmed bovine TB?
Choices; Yes/No If Yes, proceed to next question
Can you confirm these cattle were located in one of the following Southwest counties? Avon, Cornwall, Devonshire, Dorset, Gloucestershire, Isles of Scilly, Somerset and Wiltshire
Choices; Yes/No If Yes, proceed to next question
What is your date of birth?
[dd/mm/yyyy] If individual at least 18 years old, proceed to next question
Have you had a history of TB (tuberculosis) or have a close contact with a history of TB, for example a family member, close friend or work colleague?
Choices; Yes/No If No, proceed to next question
Are you currently experiencing any one of the following TB symptoms? • Persistent cough (more than three weeks) • Coughing up blood at any time • Fever • Night sweats • Unexplained weight loss • Loss of appetite • Swelling of one or more glands in the neck • Extreme fatigue and tiredness
If No, proceed to collect consent Thank you for your responses, they indicate that you are eligible to take part in the ZooTB study. We will next proceed to collecting your consent to take part in the study. If Yes, proceed to not collect consent Thank you for your responses, unfortunately we are not able to include you in the ZooTB study. To keep up with study progress and findings we will regularly update our study website (www.bristol.ac.uk/zootb)

*If prospective participant indicates they have any TB symptoms, display this message: Thank you for your responses, unfortunately we are not able to include you in the ZooTB study. You have indicated that you have at least one TB symptom, we advise that you contact your GP to discuss this with them. For further help and information visit https://www.thetruthabouttb.org.

To keep up with study progress and findings we will regularly update our study website (www.bristol.ac.uk/zootb)

Consent

Record ID
This field will be automatically populated with the study participant's unique study number (3 digits)
I confirm that I have read the information sheet dated xxxx (version xxxx) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
Please initial/check box
I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.
Please initial/check box
I understand that relevant sections of my data collected during the study, may be looked at by individuals from the North Bristol NHS Trust, where it is relevant to my participation in this research. I give permission for these individuals to have access to my records.
Please initial/check box
I understand that the information collected about me will be used to support other research in the future and may be shared anonymously with other researchers.
Please initial/check box
I agree to my General Practitioner being contacted, including any necessary exchange of information about me between my GP, the TB Service and the research team following either a positive or indeterminate IGRA blood test result.*
Provide GP details Practice name: free-text Practice address: [Town/City, County] Please initial/check box * Participants will not be able to progress through the consent form if GP details are not shared
I understand that the information held and maintained by University of Bristol and North Bristol NHS Trust researchers may be used to help contact me or provide information about my health status.
Please initial/check box
By providing my CPH number and/or farm or workplace address, I agree to data relating to the bovine TB status of my primary holding or workplace area to be accessed to help researchers measure bovine TB exposure risk.
Please initial/check box

Please note that you can still participate in the study whether or not you agree to the following statements:
I agree for an additional blood sample to be collected, stored and used in future research relating to infection and immunity. I understand that I will not personally benefit, financially or otherwise, from this donation.
Please initial/check box
I agree that my additional sample (only) may be used in future ethically-approved research involving human genetic material (DNA).
Please initial/check box
I agree to take part in the above study.
Please initial/check box
Please enter your email address so that we can send a copy of this information sheet and consent form to you
[Validated email address field]
How would you like to receive your blood test result?
Choices [Letter in the post/Email]*
***You have opted to receive your IGRA result by post, please enter your address**
[Validated address fields]
***You have opted to receive your IGRA result by email, please enter your email address if different to above**
[Validated email address field]
Same as above
Please enter your mobile telephone number. If necessary, we will use this to communicate with you about arranging your blood test.
[Validated mobile number field]

Name of Participant	Date	Signature
Dr Amy Thomas		
Principal Investigator	Date	Signature
Dr Ellen Brooks-Pollock		
Chief Investigator	Date	Signature

22.10 Questionnaire

ZooTB Study
Script for Questionnaire hosted on REDCap

Questionnaire format
The questionnaire will be hosted online with REDCap. After consenting to take part, participants will be emailed a participant-specific link to complete the questionnaire. The questionnaire will take approximately 15 minutes to complete.
If consent is obtained at the agricultural show, the questionnaire will be completed using a study tablet, again hosted on REDCap and accessed using the link provided.

The questionnaire is divided into 4 sections:

1. About you
2. Your health
3. Your work and bovine TB status
4. Your contact with cattle and farming practices

PARTICIPANT INSTRUCTIONS [to be displayed at the top of REDCap baseline questionnaire page]

Please ensure that you complete all sections before selecting 'Complete' at the bottom of the Questionnaire.

We would be very grateful if you answered all the questions, but we understand if there are some that you either prefer not to answer or are unable to answer. Please just leave these questions blank. There are no right or wrong answers.

Once you have clicked 'Submit' you will not be able to return to that section or edit your answers. If anything is unclear, please contact the **ZooTB Study Team** on 07553 785198 or zootb-study@bristol.ac.uk

1. About you
The following questions are about you, your household and your occupation

Record ID
This field will be automatically populated with the study participant's unique study number (3 digits)
What is your date of birth?
[dd/mm/yyyy]
What is your gender?
[Choices; one per line: Female, Male, Other/Prefer not to say]
What is your ethnic background?
[Multiple choice, (single answer) 1. White* 2, Mixed/multiple ethnic groups*, 3, Asian/Asian British*, 4, Black /African/ Caribbean/Black British*, 5, Other ethnic group*, 6, Prefer not to say] *Branching logic: if 1*, then 1, English / Welsh / Scottish / Northern Irish / British, 2, Irish, 3, Gypsy or Irish

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traveller, 4, any other white 2*, then 1, White and Black Caribbean/ 2, White and Black African /3, White and Asian/4, Any other mixed/multiple ethnic background 3*, then 1, Indian/ 2, Pakistani,/ 3, Bangladeshi,/ 4, Chinese,/ 5, Any other Asian background 4* then 1, African/ 2, Caribbean, /3, Any other Black/African/Caribbean background 5* then 1, Arab,/ 2, Any other ethnic group
What is your country of birth?
[Drop down]*
***What year did you move to the UK?**
Branching logic: if non-UK born selected in what is your country of birth [Drop down]
What is your current address (place of correspondence)?
Place name/number, Street name, Town/City, County, Post Code
What best describes your education level?
[Choices; one per line: No qualifications/GCSE or O Level/Diploma/A level/Degree/Higher Degree]
How many people do you live with?
[Choices; one per line: 0, I live alone, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more]
Please give the number of children you live with (≤16 years)?
[2 digits]
Please give the number of adults you live with?
[2 digits]
Have you travelled to any of the following countries in the last 12 months?
[Multiple choice, (more than one answer): Africa/South Asia/Russia/China/South America/The Western Pacific Region, including Vietnam, Cambodia, Philippines, None of the above]

2. Your health

The following questions are about your health and behaviours

Have you had the BCG (Bacillus Calmette-Guérin) vaccine?
[Choices; one per line: Yes, No]*
***Please enter when to the nearest year**
Branching logic – question only visible if selected yes to BCG [Drop down]
Rate your health on a scale of 1-100, with 1 being very poor and 100 being excellent
[3 digits]
Do you consume raw milk or raw milk products, such as raw cheese?
[Choices; one per line: Frequently, Rarely, Never]
Do you consider yourself to be at risk of exposure to bovine TB?
[Yes/No]
Do you consider yourself to be at risk of exposure to other zoonoses? For example, *Salmonella*, *E. coli*, *Campylobacter*, coccidiosis.
[Yes/No]
Do you take precautionary measures to limit the spread of zoonoses?

if yes, what are these precautions?
[Yes/No]*
***If yes, what precautionary measures do you take to limit the spread of zoonoses?**
Foot dips PPE e.g., gloves, mask Disinfecting animal housing Surveillance testing Checking for reported outbreaks in neighbouring areas Attend training and information events to help limit spread of infectious diseases Other (please give details)

3. Your work and bovine TB status

The following questions are about your occupation and the cattle you've worked with or currently work with.

What is your current job?
Farmer Farm Manager/Director Herdsman/herdswoman Milker Veterinary surgeon Veterinary nurse TB tester Slaughterhouse worker Other (please give details [free-text])
How long have you been in this occupation?
[Drop down: Less than 1 year/1-3 years/4-6 years/7-9 years/10-12 years/13-15 years/16-18 years/19-21 years/22-24 years/25+]
In the last 2 years have you worked with anyone on a regular basis from the following areas?
[Drop down, can select more than one option: Africa/South Asia/Russia/China/South America/The Western Pacific Region, including Vietnam, Cambodia, Philippines]
What best describes the type of farm you currently work on or have worked on within the last 2 years?
[Drop down: Dairy/Beef/Mixed/ I don't work on a farm/I work on multiple farms/Other (give details as free text)
What is the CPH number of this holding? *If you work on several holdings, please answer for the holding you have spent the longest duration working on.* If you are a veterinary professional (e.g., veterinary surgeon, TB tester, slaughterhouse worker) select 'Not relevant, I don't work on one holding'

Yes, I know my CPH number]/I don't know*/Not relevant, I don't work on one holding**
***What is the address of this holding?**
[Free-text]
How long have you worked on this holding?
Less than 1 year/ 1-3 years/ 4-6 years/ 7-9 years/ 10-12 years/ 13-15 years/ 16-18 years/ 19-21 years/ 22-24 years/ 25+
How many cattle are in the herd at this holding?
[Drop down: 1-10/11-50/51-100/101-200/201-300/>300]
How long have you been/were you under TB restrictions? (TB10)
[Free-text]
How many confirmed reactors have you had in this herd in the last 2 years?
[Free-text]
Have lesions been identified in the cattle you work with in the last 2 years?
[Yes/No/Don't know]
Have you worked with cattle identified with having lesions longer than 2 years ago?
[Yes/No/Don't know]
Have you worked on any cattle holdings prior to this one?
[Yes/No]*
How many cattle holdings have you previously worked on?
[Free-text]
How many of these holdings had known bovine TB disease?
[0,1,2,3,4,5,6,7,8,9,10 or more]
****What is the address of the veterinary practice or workplace that you have worked at predominately for the last 2 years?**
[Free-text]
What county and parish does this veterinary practice or workplace cover?
[Free-text]
How long have you worked at this workplace?
Less than 1 year/ 1-3 years/ 4-6 years/ 7-9 years/ 10-12 years/ 13-15 years/ 16-18 years/ 19-21 years/ 22-24 years/ 25+
How many farms with bovine TB have you worked with in the last 2 years? *If you work at one site e.g., a slaughterhouse, select 'Not relevant, I don't work on multiple farms'.*
[0,1,2,3,4,5,6,7,8,9,10 or moreNot relevant, I don't work on multiple farms*]
***How many animals with bovine TB have you worked with in the last 2 years?**
[free-text]
Do you keep any other livestock or domestic animals currently?
[Choices, one per line: Yes/No]*
***Please briefly list the species and approximate number of each**
[Free-text table headed Species and Number]

Commented [AT1]: Only show question for farming professions i.e. not veterinary professionals.

Commented [AT2]: Only show question for farming professions i.e. not veterinary professionals.

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Commented [AT9]: Only show question for veterinary professionals.

Commented [AT10]: Only show question for veterinary professionals.

Commented [AT11]: Only show question for veterinary professionals.

4. Your contact with cattle and work practices

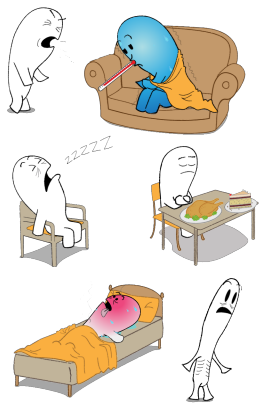
The following questions are about the types of contact you have with cattle and certain farming practices. Please continue to answer for the holding you described in the previous section 'Your work and bovine TB status'

How often do you have close or physical contact with cattle?
[Drop down: Daily/Weekly/Monthly/Rarely/Never]
How many hours a day do you have contact with cattle?
[2 digits]
Where do you spend the most amount of time with cattle?
[Choices, one per line: Inside, Outside, Both inside and outside]
What best describes your day-to-day contact with cattle?
[Drop down: Direct contact in a closed space, Direct contact in an open space, No direct contact with cattle]
Select the practices where you feel you have the closest contact with cattle?
[Drop down, more than one can be selected: Milking, Calving, Feeding, Herd health management procedures, Other (describe free-text]
Are high pressure hoses used in the environment you work in with cattle?
[Choices, one per line: Frequently*, Rarely*, Never]
***Is this mostly inside or outside?**
Branching logic – question only visible if selected 'Frequently' to using high pressure hoses. [Choices, one per line: Inside/Outside/Both inside and outside]*
Have you knowingly had contact with animals infected with TB other than cattle? For example, this could be physical contact with animals or contact with the environment they live in, such as a garden or house.
[Yes/No]*
***Please briefly list the species and approximate number of each**
[Free-text table headed Species and Number]

22.11 Leaflet to be given to prospective individuals who are identified to have TB symptoms in pre-screening questionnaire

TB, covid-19 and long-covid symptoms checker

TB

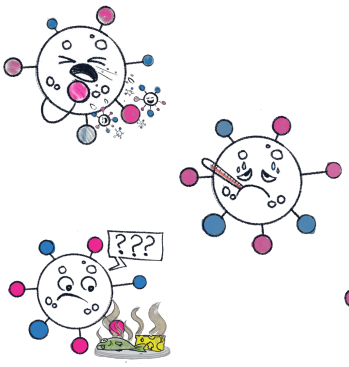


Tuberculosis (TB) is caused by a bacteria known as mycobacteria-tuberculosis.

The most common symptoms of TB are a cough, fever, weight loss, loss of appetite, tiredness, and night sweats. TB symptoms become worse over time.

For more about TB, visit www.thetruthabout**tb**.org

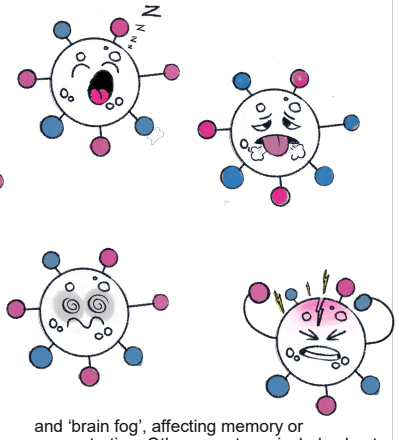
Covid-19



Covid-19 is caused by a type of virus known as a coronavirus.

The most common symptoms of covid-19 are a cough, fever and changes to sense of smell or taste. Long-covid is still poorly understood but is thought to involve a number of symptoms. The most common are an ongoing cough, long term changes to smell and taste, fever, tiredness, breathlessness, headache

Long-covid



and 'brain fog', affecting memory or concentration. Other symptoms include chest pain or tightness, difficulty sleeping, heart palpitations, dizziness, pins and needles, joint pain, depression and anxiety, tinnitus, earaches, feeling sick, diarrhoea, stomach aches, loss of appetite, headaches, sore throat and rashes.
For more about covid-19 visit: www.nhs.uk/conditions/coronavirus-covid-19