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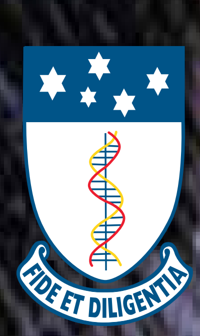
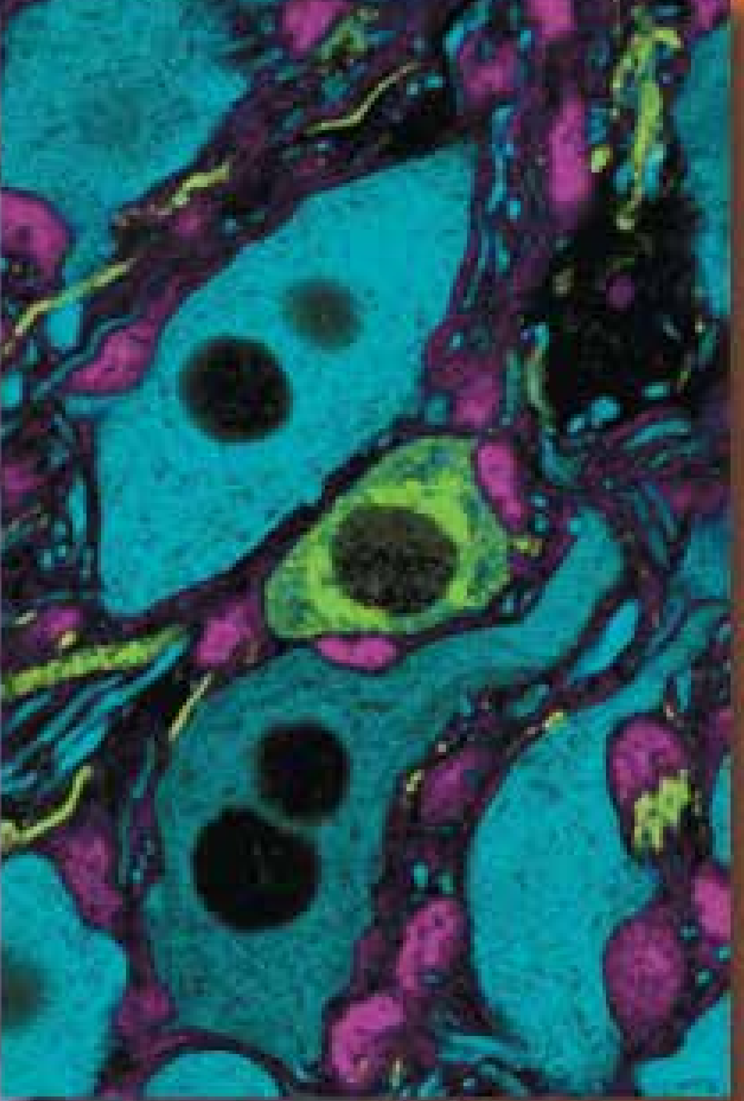


AUSTRALIAN JOURNAL OF Medical Science 2024



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LETTER TO THE EDITOR

ORIGINAL ARTICLES

The impact of COVID-19 on blood transfusion services at Colonial War Memorial Hospital, Suva, Fiji

Strategies for isolating microbiota from faecal samples: are all gut microorganisms potentially cultivable?

REVIEW ARTICLE

Heparin induced thrombocytopenia: diagnosis, treatment, challenges and future of testing

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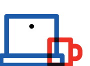

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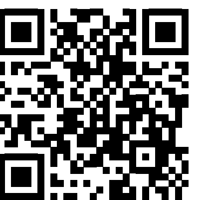

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LETTER TO THE EDITOR

To the Editor,

We are writing regarding the article "*Who is educating our future Medical Laboratory Scientists? A perspective of AIMS accredited Medical Laboratory Science programs in Australia*", published in the May & August edition (Vol. 43, 2022) of the Australian Journal of Medical Science (AJMS). As a group, we have taken time to consider the findings of this study and commend the authors for attempting to answer important questions for the profession. However as some of the academics responsible for the education of our future medical laboratory scientists (MLS), we are deeply concerned about the study design and misleading conclusions made. We feel that the findings present academics teaching into MLS programs in a negative light and may lead to concerns for employers (of our graduates), other medical scientists, recent graduates and current and potential students, about who is teaching our future MLS and the quality of university education provided by AIMS accredited undergraduate Medical Laboratory Science and Laboratory Medicine programs.

Using a voluntary, online survey the study sought to collect quantitative and qualitative data in order to answer the following questions:

1. What are the academic qualifications, teaching and MLS experience of MLS academic staff in Australia from 2019-2021?
2. What are the numbers of students enrolled and graduated in MLS programs in Australia from 2019-2021?
3. What are the employment outcomes for MLS graduates from 2019-2021?

For the first question the study reported staff from ten AIMS accredited programs completed the survey (31 academics) and responses from one institution account for 29% of respondents (n=9 academics). This is a small sample group compared to the total number of academics teaching into MLS programs in Australia. In regard to academic qualifications, content expertise, teaching and leadership/coordination, the study does not discriminate between the requirements for foundation and professional subjects defined in the AIMS Accreditation Standards and Procedures (AASP) (Table 1). Approximately two thirds of the academic-related data and subjects included in the study are for foundation subjects (usually completed in first and second year of an AIMS accredited program) and they should be excluded. For those subjects, there is no requirement they be coordinated and taught by academics with MLS qualifications and/or experience in pathology laboratories. The AASP recognises these subjects are common to biomedical and allied health degrees and MLS students study these subjects with students from non-MLS programs, which are coordinated and taught by academics with appropriate qualifications in the field (e.g. statistics, chemistry). The inclusion of that data and the academics teaching those subjects, has resulted in a significant over-representation of academics who do not require MLS backgrounds and the data and findings published in the article should not be used to draw conclusions about who is teaching our future MLS.

***Table 1**. Foundation and professional subjects for undergraduate Bachelor MLS programs as defined in the AIMS Accreditation Standards and Procedures (AASP v2020) when the study was performed. This subject list remains current and unchanged.*

Foundation Subjects	Professional Subjects
Biochemistry	Anatomical Pathology/ Histopathology (including where possible, Cytopathology)
Cell and Tissue Biology (including Anatomy, Physiology and Histology)	Chemical Pathology
Chemistry	Genomic Pathology
Genetics	Haematology (including Haemostasis)
Immunology	Immunopathology
Microbiology	Medical Microbiology
Molecular Biology	Pathophysiology (general/ systemic pathology with emphasis on the relationship between disease process and laboratory diagnostic techniques)
Statistics	Transfusion Science
	Work Integrated Learning (Professional Practice, aka, Clinical Placement)

In contrast, the professional subjects (discipline specific) defined in the AASP (Table 1) do require the academic and/or teaching staff to have qualifications and experience

in MLS and diagnostic pathology. Where universities do not have a suitably qualified academic on staff to coordinate and teach a subject, an alternate academic can be appointed to coordinate the subject, provided, the design, learning and assessment is informed and taught by external staff or partners, who are actively employed or engaged in medical science (pathology) and undertake ongoing professional development. These staff or partners can hold academic, technical, part time, sessional, casual, adjunct, industry partner and/or honorary positions. Unfortunately, the study does not capture the significant contribution these individuals make in all MLS programs and only presents data from academics for five subjects defined professional for accreditation purposes. For those five subjects, it was not possible to determine how many were from the same institution and/or taught within teams from the data presented. This represents <10% of the academics and professional subjects in all AIMS accredited programs, who were active and teaching at the time the study was conducted. It is a small sample and should not be used to draw conclusions about all the academics teaching our future MLS.

The second question the study sought to answer was how many students were enrolled or graduated from MLS programs in Australia from 2019-2021. For this question, data was presented and discussed from seven AIMS accredited programs. We agree with the authors that care must be taken interpreting the numbers and drawing conclusions, particularly for 2020 and 2021. This is in part due to the incomplete data set and timing of the study when all universities were impacted by the COVID-19 pandemic. Enrolment numbers were also collected mid-year 2021 and do not reflect the final number of students still active at the end of the year, and the authors did highlight this in the study limitations. Unfortunately, the total number shown does not allow for the differentiation of changes in internal enrolment, stage of study (year level or load) or the influence of leave of absence applications, withdrawals, deferral of studies or subsequent return to study that occurred. Nor does it capture the influence of commencing students, who deferred their offer to start university in 2020 and 2021, when teaching was either fully online or via hybrid classes. This impact was experienced and reported by many universities and non-MLS programs in Australia and world-wide.

University and region-specific circumstances can also explain the impact to enrolment numbers at the time the study was performed. The Queensland-based universities in particular (Griffith, JCU, QUT), all experienced a one-off decrease in 2020 commencing enrolments (approximately one third), due to the reduced intake that coincided with the move of Grade 7 from primary to secondary school, state-wide. This was reported by all Queensland universities, not just those with AIMS accredited MLS programs. In 2021, all universities in Australia also reported a pause and/or decrease in international student enrolments, when new and returning students were unable to enter the country when the borders closed for international travel. We would also like to clarify that the overall 12.1% growth reported in the article for 2021 is not due to the new program at Charles Sturt University (CSU), and that is a misinterpretation of the enrolment numbers CSU provided. When CSU introduced their new program structure, commencing (new) and continuing students were all transferred (enrolled) into the new program, and the increased numbers noted represent an internal CSU growth (14% or n=9 students). Considering this and that Queensland universities account >50% of the numbers in the study, we query the accuracy of the growth rates calculated, as well as the author's call for a longitudinal study to investigate limited program growth based on the rate this study reported (0.8%). There was also no data collected or evidence presented to support the suggestion students were springboarding from MLS into other health or medical programs, and that statement should be interpreted as the author's opinion, not a finding investigated or supported by the study.

The third question the study sought to answer was how many graduates obtained employment (part, or full time) during the same time period. For this question we would like to draw the reader's attention to the figures presented for each program (Table 1 in the published article). Other than 2020 for QUT and RMIT (who were particularly impacted by the Melbourne lockdowns), all programs report ≥75% graduate employment, with most reporting near or 100% full employment annually. These are good outcomes for the graduates and indicate the majority have secured employment before they finish university and/or find work within weeks or months of graduating. As such, we are confused by the reasoning for the need for "a focussed plan to improve retention of MLS graduates and provide better support for the profession to improve graduate employability is required by both the university and AIMS". This again appears to be author's opinion and the study design and data presented did not investigate nor provide evidence to support this statement. Communication with those involved in AIMS accredited programs through various forums would confirm that all programs report similar graduate outcomes, including those that did not take part in this study. The reporting and discussion of year to year enrolments, student demographics and graduate outcomes to our industry and laboratory partners occurs at all Program Advisory Committee (PAC) meetings held by AIMS accredited programs. The PAC is composed of at least 75% external members (e.g. senior and/or supervising scientists, laboratory managers, workplace

training and professional placement coordinators) representing central, metropolitan, regional and rural pathology laboratories for all disciplines (if possible), along with an academic(s) from another AIMS accredited program and recently graduated and current students.

With regard to the final conclusions, unfortunately the knowledge attained and published from the study has not provided a better understanding of who is teaching our MLS students. We wish to take this opportunity to make clear that all MLS programs with (or seeking) AIMS accreditation are required to provide resumes, work history and pathology laboratory experience details for all staff who teach into and/or contribute to the learning design of MLS programs (holding academic, technical, MLS, full time, part time, sessional, casual, adjunct, industry partner or honorary positions). Accredited programs are also required to report changes to professional subject teaching staff in annual program reports and during on campus accreditation/reaccreditation visits. This allows the AIMS Accreditation Panel to continually assess, monitor and ensure standards are being met and appropriately qualified people are teaching our students.

We acknowledge the authors have sought to answer important questions with this study and published findings; however, both should be interpreted with care. There are issues and limitations associated with the timing of the survey and low participation rate, leading to a small sample size and inaccurate enrolment numbers. This has resulted in misleading statements and conclusions about the enrolment numbers and who is teaching our MLS students. Redesigning (and redeploying) the survey and study to differentiate foundation and professional subjects and capture the contribution our industry- and laboratory-based partners and staff make. A more detailed investigation in enrolments, study loads, year levels and decisions students made regarding their studies (deferral, leave of absence, withdrawal) and/or completing and graduating in 2020 and 2021 based on Academic Census and end of year numbers, would better answer the questions this study sought to investigate.

Sincerely,

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The impact of COVID-19 on blood transfusion services at Colonial War Memorial Hospital, Suva, Fiji

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Fiji National University, School of Health Sciences, Department of Pathology and Medical Laboratory Science, Suva, Fiji

Abstract

The aim of this study was to find the impact of COVID-19 on transfusion services at Colonial War Memorial Hospital, Suva. The objectives were to identify trends in blood donation and its management before and during the pandemic, and to look at strategies for recruiting donors during the pandemic.

The data collected demonstrated an 8.8% (n=690) decline in donations between the years 2019 to 2020 with a 4.69% (n=336) decline in donations between 2020 to 2021. The findings suggest that the major cause of the decline was due to COVID-19 lockdowns and movement restrictions. Demographics e.g. age of donors were also evaluated to find blood donor deferral and age of donors with the findings showing the age category of 16-45y to be common for deferrals as well as for donation.

A strategic approach needs to be formulated for scenarios like the COVID-19 pandemic to implement working policies.

Keywords: COVID-19, blood donations, deferrals

Introduction

Background

In December 2019, the first cases of coronavirus disease were reported in Wuhan, China and after countries around the world started reporting similar cases, a global pandemic was declared by the World Health Organization (Chen *et al* 2020). Increases in the case numbers led to various restrictions and measures being imposed. The imposed restriction impacted various sectors including health and one department in particular was the blood service department. The impact COVID-19 had on blood services was quite evident in Saudi Arabia as a severe disruption in blood supply and demand was noted in the five major cities (Hakami *et al* 2020). The impact faced was reported to be led by poor planning and management of blood stocks during the pandemic (Gehrie *et al* 2020). The adoption of new strategies in a resource-constrained blood center can lead to well-managed blood stock levels and an improvement in blood supply (Biswas *et al* 2021). Fiji, like other Pacific countries, is resource-constrained and when pandemics like COVID-19 strike, a major burden falls on the transfusion service. To address the issues of blood supply and demand, new strategies and policies need to be adopted.

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In March 2020, Fiji reported its first COVID-19 case, and as case numbers increased, restrictions and lockdowns were imposed. The country had two major lockdowns – one in 2020 and the other in 2021 (Ministry of Health and Medical Services, 2022). The restrictions greatly impacted the health sector. The country's largest medical facility, the Colonial War Memorial Hospital (CWMH), carries out numerous surgeries and transfusions daily. The blood required for these relies solely on the blood service department at CWMH. The constraints the blood services faced during the pandemic can be shown through the data available on the blood supply and demand within the period of the pandemic.

Blood donations save countless lives, however when pandemics like COVID-19 strike, the significant impact is faced by the blood service department along with the challenges that follow. To understand the magnitude of this impact, this study evaluated trends in blood donations while looking at the adoption of blood management policies and changes to donor recruitment. This study also evaluated common deferral causes along with donor

demographics to potentially target the population for donor recruitment that could be undertaken during similar scenarios like the COVID-19 pandemic.

Aim

A systematic review by Chiem *et al* (2022) was conducted to find the impact COVID-19 had on blood transfusion services worldwide (Chiem *et al* 2022). Thirty-eight articles were reviewed and a significant decrease in blood donors was noted throughout the different regions of the world. The impact of COVID-19 on blood stocks around Fiji was highlighted in a news article in 2022 which described the challenges faced by blood services in donor recruitment as the pandemic kept donors away and the non-government organization, the Red Cross, had to intervene to help create awareness on the blood shortages faced (Critical blood shortage in Fiji, 2022).

While there is currently no available data or publications that can show the impact COVID-19 had on the blood stocks in the country, a comparative analysis of data from the pre-COVID phase and during the COVID phase will supply such evidence of the impact COVID-19 had on blood transfusion services at CWMH. This study evaluates the deferral reasons to find the common causes, and looks at strategies to undertake in response to this. The study also highlights the adoption of strategies that were taken in recruiting donors during the pandemic and how effective these strategies were. Lastly the study examines the major issues faced during the pandemic and recommends how these issues can be addressed to help prepare for a similar scenario.

Objectives

1. To determine the blood donation trends at CMWH before and during the pandemic.
2. To compare the management of blood components at CMWH before and during the pandemic.
3. To identify donor recruitment strategies undertaken during the pandemic at CMWH.

Methods

Collection of primary data

Approval of the research proposal from the College Human Health Research Ethics Committee (CHHREC) was undertaken. Upon approval from CHHREC (ID:036.22), the consent of the currently serving blood service staff for voluntary participation in the interview was gained. The staff being interviewed were given an information sheet (Figure 1) and reassured that their names were not used in this research; instead, a unique code was given. The participants had the option of opting out at any time during the interview. The interview was conducted in the presence of the supervisor. The feedback collected through the interview questionnaire was compiled and evaluated in excel spreadsheets.

Collection of secondary data

The secondary data were collected in three separate log sheets for component usage, donation trend, and donor deferrals and this was also approved (CHHREC ID:036.22).

The data from the log sheet were entered into excel spreadsheets and checked for duplication. The data were analysed and used to evaluate the chi-square to find significance.

The analysed data were interpreted as below:

1. The data from blood services were used to determine the demographics, such as the age and gender, of donors during and before the COVID-19 Pandemic (January 2019–March 2022) while looking at the trend in donations.
2. Data collected on the donor deferral rates were used to show the common reason for deferrals during the pandemic (March 2020–March 2022).
3. The data collected from the blood bank were used to determine the usage of blood components: red blood cells, platelets, fresh frozen plasma, and cryo-precipitate during and before the COVID-19 pandemic (January 2019–March 2022).

The inclusion and exclusion criteria are shown in Table 1.

Study design

Mixed methods, which includes a cross-sectional and qualitative study.

Study setting

The study was conducted in the blood service and blood bank departments at CMWH.

Study population or sample

The data were collected from registers available in the blood service and blood bank departments, and an interview was carried out with the blood service staff.

Ethical consideration

Data collected was stored in an encrypted file on a USB; the USB was kept with the primary supervisor. All donor information collected is kept confidential. No donor name was used; instead, a unique code was given to each donor. There was no intervention or direct contact with donors as all the data were collected from the registers.

INTERVIEW INFORMATION SHEET

Research Title: The impact of COVID-19 on blood transfusion services at Colonial War Memorial Hospital Suva

Primary Investigator: Nishal Murthi

The details of the study are provided as below. If there are any clarifications or queries on any part of the study which is unclear you can ask me for the clarifications.

Purpose of Research

I wish to carry out an interview on your perspective on blood donor recruitment and the challenges faced during the COVID-19 pandemic. The information will be used to address the objective on strategies implemented and impact faced by the blood donor services during the pandemic.

Participant Selection

You are selected for this interview of being a blood service staff and having a first-hand encounter with donors during and prior to the pandemic.

Voluntary Participation

It is up to you to decide whether or not to take part. If you do decide to take part and approve in taking part in the interview, you will be given this information sheet to keep and be asked to sign a consent form. You are free to withdraw at any time and without giving a reason.

Risks

There is no risk involved. The interview sheet used will not have your name; instead a unique code will be given.

Benefits

The information provided by you in the interview will help in identifying the gaps and challenges faced during the pandemic in blood services and also help in describing the blood donor turnout trend.

Confidentiality

The information collected from this research project will be kept confidential. Your names will not be used in this research. The information provided will be kept with me and my research supervisors in a locked cabinet and will only be used as the intended research.

Thank you

***Figure 1**. Interview information sheet.*

***Table 1**. Inclusion and exclusion criteria.*

Inclusion	Exclusion
• Data from January 2019–March 2022 was collected. • Interview of blood service staff was Conducted.	• Data before January 2019 was not used. • No interview of donors was conducted.

Confidentiality

All interviewees' names were kept confidential and were not used in the study. The data collected will be stored in a locked cabinet for 7 years, after which the data will be shredded.

Results

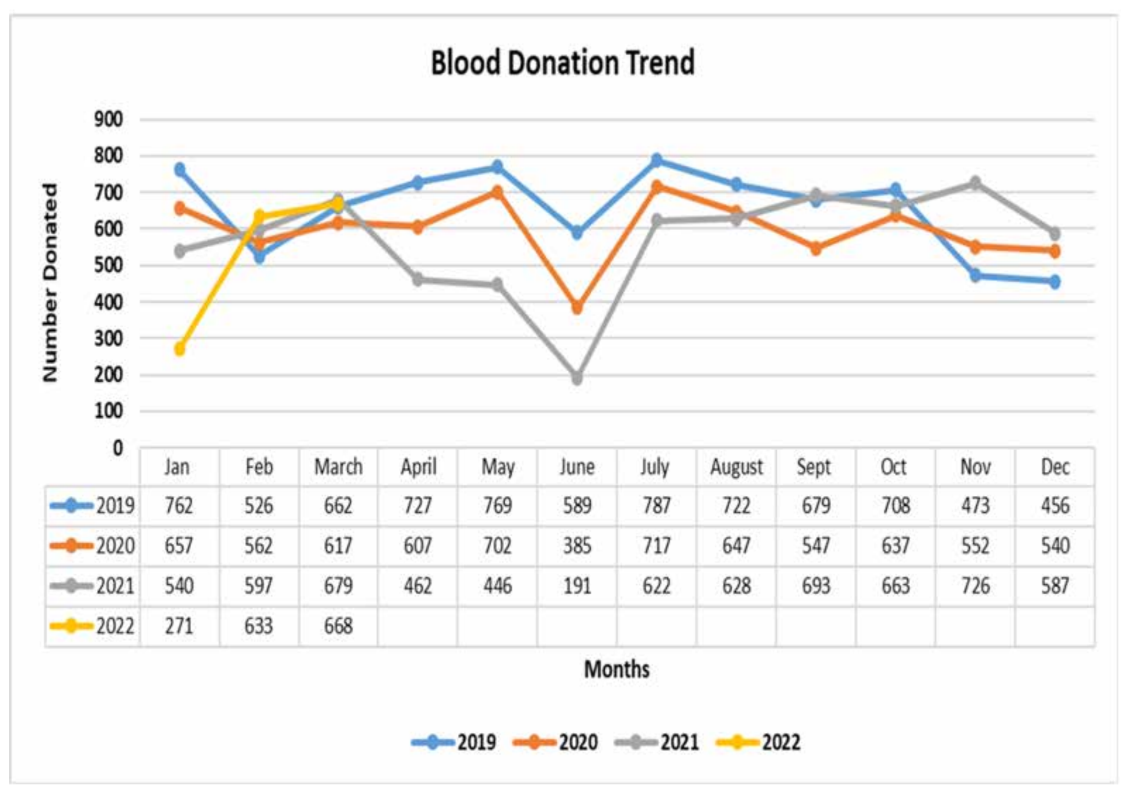
Blood donation trend

A total of 23436 blood donations were recorded from January 2019 to March 2022. The data demonstrated a decline of 8.8% (n=690) in donation between the years 2019 (n=7860) to 2020 (n=7170) and a 4.69% (n=336) decline in donation between 2020 to 2021 (n=6834) as restrictions were imposed upon the increase of COVID-19 cases in the country.

The re-emergence of COVID-19 in the community caused lockdowns and donations of blood declined from March 2021 as the blood services faced a critical low.

June has shown to be the month with the least donations throughout the years (Figure 2). A notable change in donation was seen as of July 2021 with the uplifting of certain borders and hospitals returning to normal operation with rescheduled surgeries. The contingency plan for recruiting donors showed promising results as more donors stepped in to donate.

Blood donations increased after January 2022 (n=1572) this was a result of the uplifting of complete lockdowns and a gradual return to normalcy (Figure 2).



	Jan	Feb	March	April	May	June	July	August	Sept	Oct	Nov	Dec
2019	762	526	662	727	769	589	787	722	679	708	473	456
2020	657	562	617	607	702	385	717	647	547	637	552	540
2021	540	597	679	462	446	191	622	628	693	663	726	587
2022	271	633	668									

***Figure 2**. Blood donation trend: January 2019–March 2022.*

Blood component usage

A decline in all blood component usage was noted each year as the outbreak of COVID-19 led to a decrease in elective surgeries. The limited supply in blood components led to the prioritization of components to the treatment of patients depending on need.

An 89% (n=641) reduction in packed red cells were noted between 2019 (n=7233) to 2020 (n=6592) and an 88% reduction (n=581) was noted between the periods of 2020 to 2021 (n=6011). Red cell components show the most decline in usage from the month of May to July 2021 as this was commonly used in elective surgeries that had to be rescheduled due to the pandemic.

Platelet concentrate showed a 28% (n=460) decline between 2019 (n=1620) to 2020 (n=1160) however there was an increase of platelet use of 0.09% (n=1) between the years 2020 to 2021 (1161). Platelet usage did not show a decline during the pandemic period as the usage increased slightly from the previous year.

Fresh frozen plasma showed a reduced use of 20% (n=343) between 2019 (n=1739) to 2020 (n=1396) and a 18% (n=245) reduced use between 2020 to 2021 (n=1151). Cryoprecipitate showed a 30% (n=136) decline in usage from 2019 (n=451) to 2020 (n=315) and a 19% (n=60) reduced use from 2020 to 2021 (n=255). Both fresh frozen plasma and cryoprecipitate showed a decline in usage during the pandemic period with the reduced supply components prepared on as need basis. The decline was noted due to the lockdowns imposed and the rescheduling of most elective surgeries. However, all blood component usage tends to increase after January 2022 as blood donation returns to pre-pandemic level (Figure 3).

Donor deferral: March 2020-March 2022

A total of 2066 deferrals were recorded during the pandemic period from March 2020 to March 2022.The deferrals were categorized into three causes; physical, history and personal as shown in Table 2.

Physical causes were the main reason for most deferrals during this period. The common deferrals due to physical causes comprised of donors having low haemoglobin levels and low or high blood pressure. Low haemoglobin was noted to be slightly common in the female donor population. In contrast, low and high blood pressure was shown to be more common in the male donor population.

Donors on medication was the common reason for deferral in the history cause as this comprised of individuals taking medication for chronic conditions like diabetes, high blood pressure and with some taking medication for flu-like symptoms.

Personal causes varied from self-deferral, donor not having enough rest or donor did not have a meal before donation. These were due to lack of awareness on the criteria required for blood donation and this prevented potential donors from donating.

The highest number of deferrals were recorded in 2021, as in this year COVID-19 cases peaked in the central division (Table 3) Uncertainty and fear set in, fewer donors turned up, and restrictions brought a halt to donations, and most donations were through mobile drives in unrestricted areas.

Donor demographics

The following figures show the number of donations of the two genders against their age categories. Age categories between 16-25, 26-35 and 35-45 have shown to be the common categories for most donation in both genders when compared to age categories 46-55 and 56-65. The following results indicate that donation was common in the younger to the middle age category regardless of gender.

Males tend to show a higher donation of blood compared to female donors and this may be a result of females being deferred mostly due to the low haemoglobin (Table 2).

Figures 5-7 demonstrate the donations trend by age during and before the pandemic and as shown in the figures, a significant decline of donations was noted in all age categories following March 2021 as restriction were imposed and COVID-19 cases started to increase.

The male donor population recorded its highest number in blood donations in March 2021 across all age categories. This was due to scheduled elective surgeries; however, after the announcement of community outbreaks of COVID-19, blood donations decreased drastically. A steady decline was noted on the female donor population across all age groups as the cases peaked.

There was a significant peak in the age category between 56-65 in June 2019 as this shows the highest blood donation of the male population and this was due to the recruitment of healthy donors for family-based replacements of donor units.

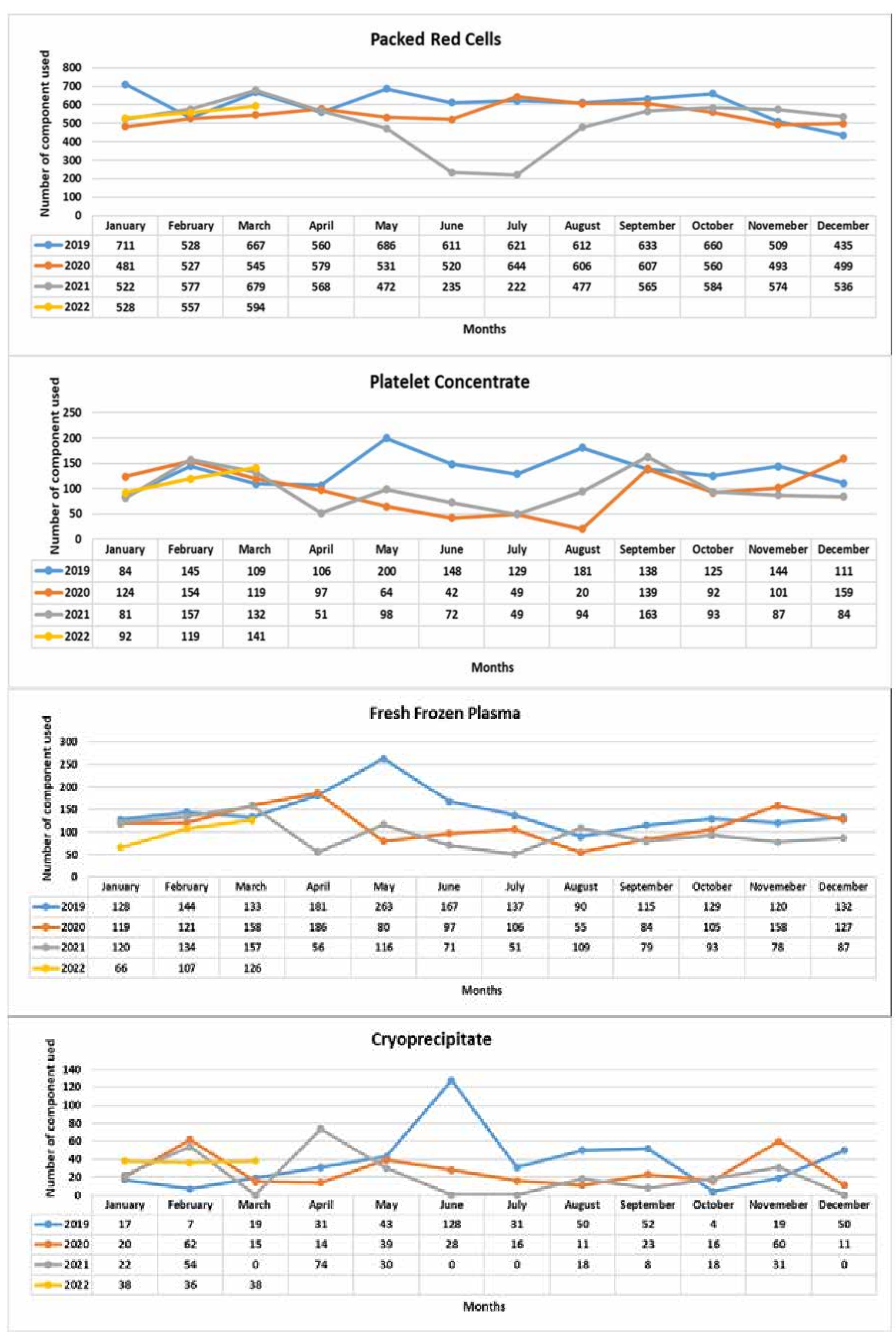


Figure 3. *Blood component usage January 2019 to March 2022.*

__Table 2__. Causes of deferral.

Personal Cause			
	2020	**2021**	**2022**
Did not have a meal	8	8	2
Did not have enough rest	10	4	0
Difficult vein	1	2	2
Fasting	0	0	1
Feeling weak	0	0	1
Over age limit	2	0	1
Self-deferred	7	8	3
Total	**28**	**22**	**10**

History Cause			
	2020	**2021**	**2022**
Abscess	7	10	0
Accident	0	1	0
Admitted	4	0	0
Allergy	1	3	0
Asthma	10	6	2
Attending medical clinic	2	0	0
AVI	1	0	0
Blisters	2	0	0
Bloody stool	1	0	0
Breast feeding mum	3	7	1
Bruise	0	0	1
Chest pain	2	1	1
Chronic cough	21	6	6
Cold	1	0	1
Cold sores	0	1	0

Consumed alcohol	0	4	0
Diabetic	4	4	2
Drugs	1	0	0
Ectopic pregnancy	0	1	0
Eye infection	0	1	0
Fish poisoning	1	0	0
Gout	1	0	0
Heart problem	0	1	0
History of transfusion	1	0	0
HTN	0	2	0
Infected wound	0	1	0
Infectious disease	15	2	2
Injury	1	2	3
Knee swelling	0	1	0
Lapse in menstruation	0	21	8
Last donation less than 3 months	12	6	1
Lesion on finger	1	0	0
Liver abscess	1	0	0
Lump	1	0	1
Medical condition	11	1	0
Menstruation (not 2 weeks)	31	41	15
Mild stroke	1	0	1
Miscarriage	1	0	0
Not ABO blood group specific	23	17	10
On antibiotic	4	6	0
On medication	68	24	11
Open wound	33	12	5
Piercing	2	2	2
Pregnant	1	1	0

Pregnant delivered less than 6 weeks	1	0	0
RHD	1	1	0
Ringworm	1	0	1
Sinuses	1	0	0
Skin disease/rash	23	8	2
Sore throat	2	0	2
Surgery	3	2	0
Swabbed for COVID	0	1	0
Swollen LEG	0	2	0
Symptomatic of COVID	0	0	1
Tattoo (less than 6 months)	14	12	5
Tooth extraction	1	0	0
Travel history	2	0	0
Underwent medical treatment	0	1	0
Total	**318**	**212**	**84**

Physical Cause			
	2020	**2021**	**2022**
Body pain and fatigue	1	0	0
High blood pressure	90	181	80
High blood pressure and low Hb	0	1	0
High blood pressure and on medication	0	1	0
High body temperature	1	0	0
High temperature and low Hb	0	1	0
Low blood pressure	91	77	43
Low Hb	290	355	120
Low Hb and High blood pressure	0	8	12
Low Hb and Low blood pressure	7	9	0

Overage limit	0	5	0
Underage limit	0	2	0
Underweight	5	7	0
Vein collapsed	2	3	0
Total	**487**	**650**	**255**

Table 3. *Number of deferred donations by year and cause.*

Causes	**2020**	**2021**	**2022**	**Total**
Personal	28	22	10	**60**
History	318	212	84	**614**
Physical	487	650	255	**1392**
Total	**833**	**884**	**349**	**2066**

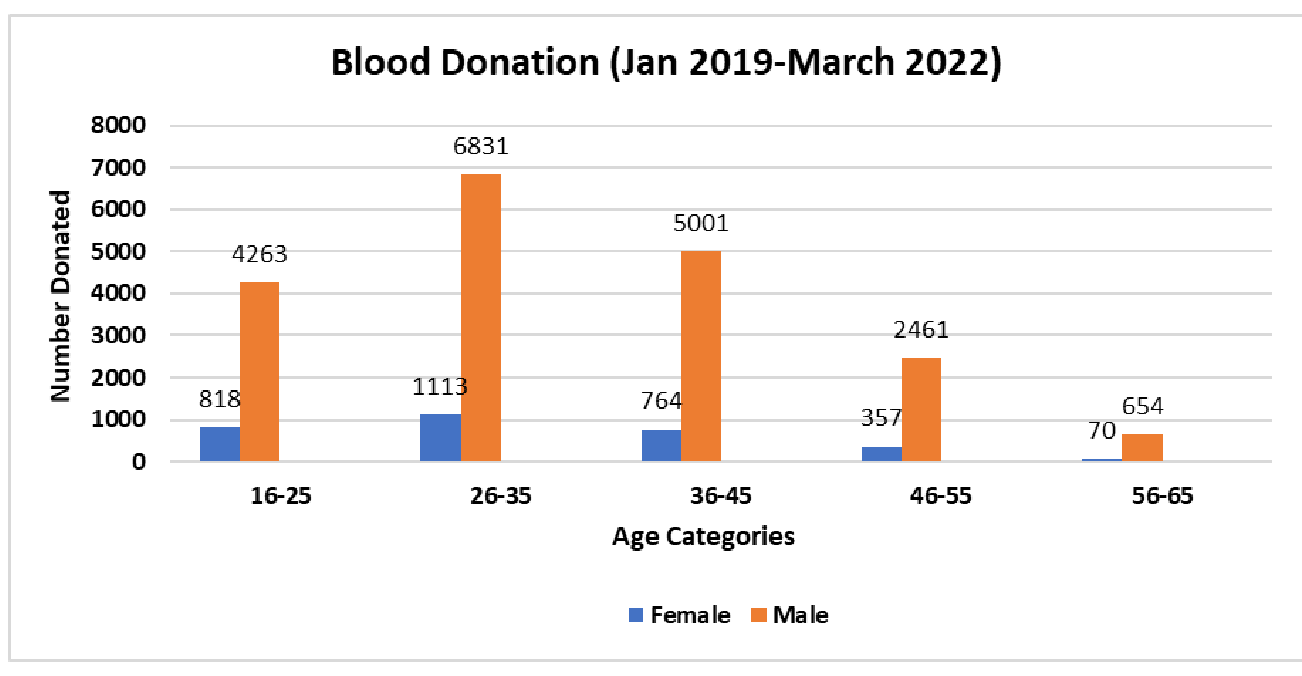
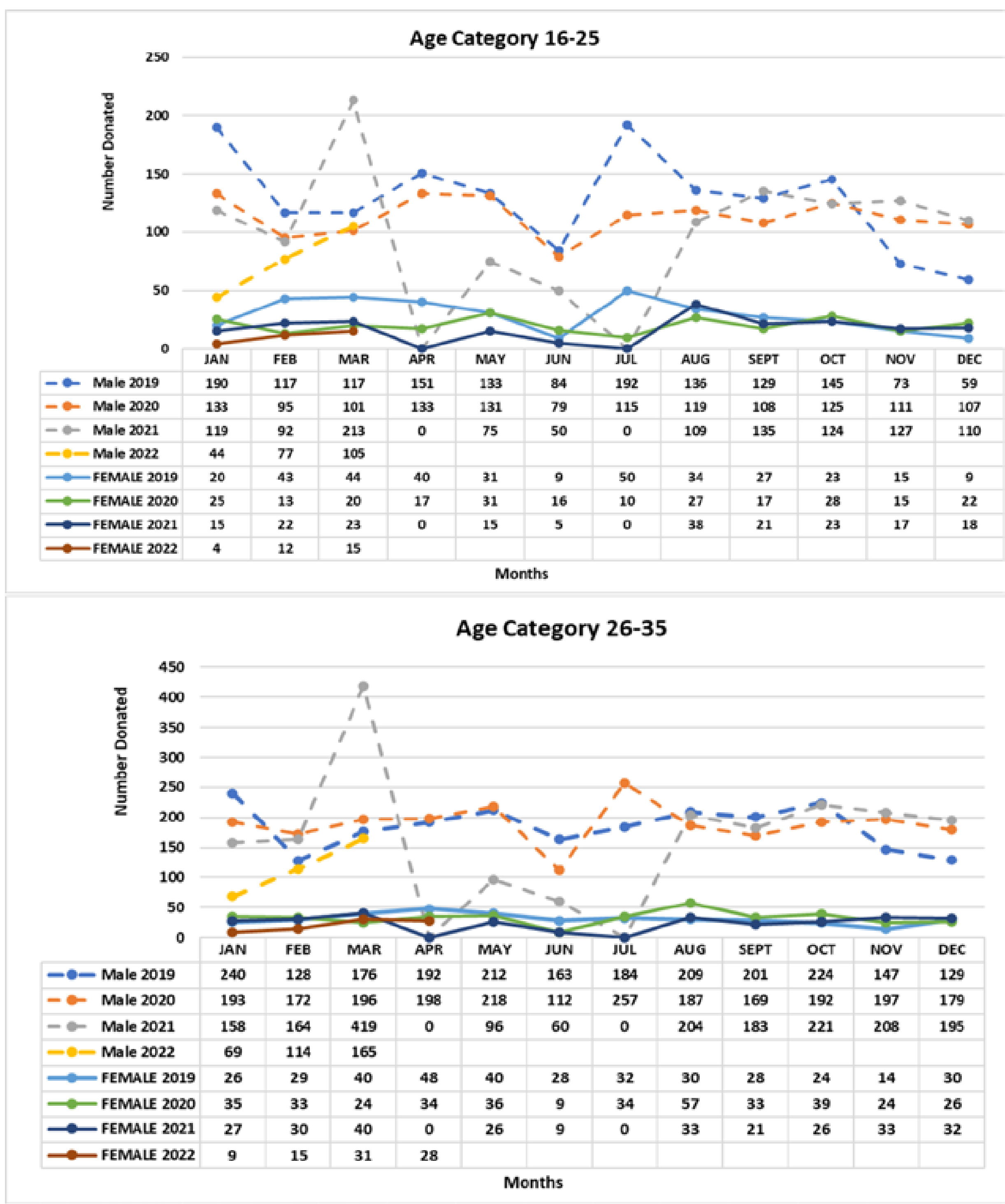


Figure 4. *Donation age and gender category (January 2019-March 2022).*



	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
Male 2019	190	117	117	151	133	84	192	136	129	145	73	59
Male 2020	133	95	101	133	131	79	115	119	108	125	111	107
Male 2021	119	92	213	0	75	50	0	109	135	124	127	110
Male 2022	44	77	105									
FEMALE 2019	20	43	44	40	31	9	50	34	27	23	15	9
FEMALE 2020	25	13	20	17	31	16	10	27	17	28	15	22
FEMALE 2021	15	22	23	0	15	5	0	38	21	23	17	18
FEMALE 2022	4	12	15									

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
Male 2019	240	128	176	192	212	163	184	209	201	224	147	129
Male 2020	193	172	196	198	218	112	257	187	169	192	197	179
Male 2021	158	164	419	0	96	60	0	204	183	221	208	195
Male 2022	69	114	165									
FEMALE 2019	26	29	40	48	40	28	32	30	28	24	14	30
FEMALE 2020	35	33	24	34	36	9	34	57	33	39	24	26
FEMALE 2021	27	30	40	0	26	9	0	33	21	26	33	32
FEMALE 2022	9	15	31	28								

***Figure 5**. Age category donations 16-25y and 26-35y.*

Age Category 36-45

Number Donated

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
Male 2019	122	99	139	150	176	101	170	162	155	143	110	111
Male 2020	157	143	147	116	154	95	163	146	127	126	114	129
Male 2021	142	155	307	0	69	33	0	122	157	157	147	109
Male 2022	69	114	165									
FEMALE 2019	19	18	23	23	36	12	31	27	30	24	17	18
FEMALE 2020	20	22	23	29	12	26	22	22	24	17	10	
FEMALE 2021	8	16	22	0	20	3	0	32	25	19	22	29
FEMALE 2022	8	11	21									

Months

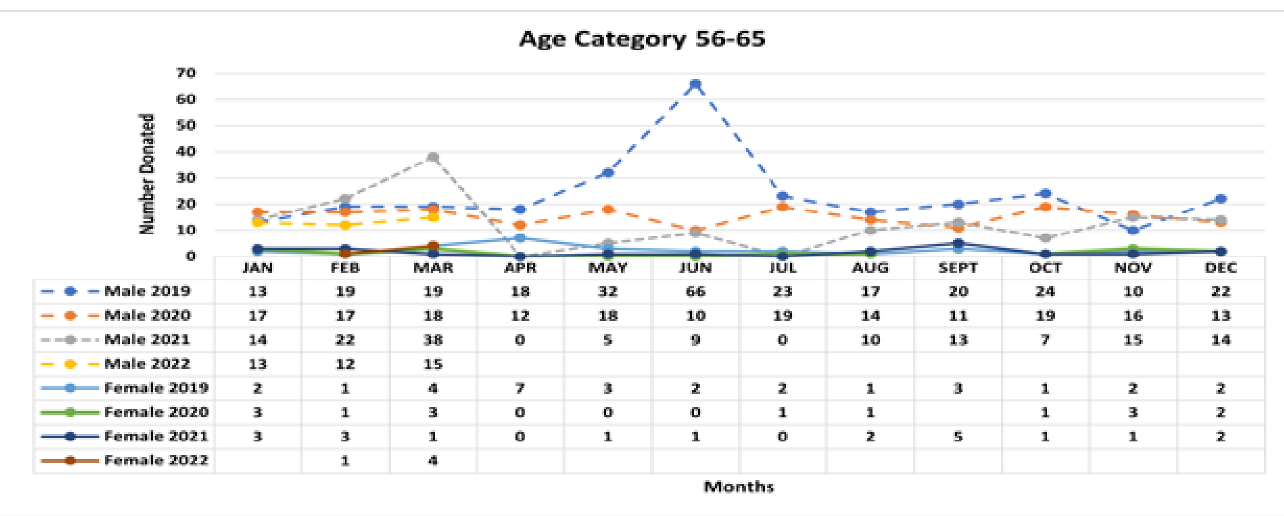
Age Category 46-55

Number donated

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
Male 2019	100	53	85	73	85	99	76	83	75	84	76	59
Male 2020	65	61	75	64	65	43	80	59	54	71	47	49
Male 2021	43	79	171	0	29	18	0	47	63	60	63	54
Male 2022	24	49	80									
Female 2019	9	13	8	15	9	6	14	19	9	10	8	14
Female 2020	9	6	11	10	13	8	11	10	6	9	6	2
Female 2021	8	10	12	0	6	2	0	13	12	11	12	10
Female 2022	4	10	12									

Months

__Figure 6__. Age category donations 36-45y and 46-55y.



	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
Male 2019	13	19	19	18	32	66	23	17	20	24	10	22
Male 2020	17	17	18	12	18	10	19	14	11	19	16	13
Male 2021	14	22	38	0	5	9	0	10	13	7	15	14
Male 2022	13	12	15									
Female 2019	2	1	4	7	3	2	2	1	3	1	2	2
Female 2020	3	1	3	0	0	0	1	1		1	3	2
Female 2021	3	3	1	0	1	1	0	2	5	1	1	2
Female 2022		1	4									

__Figure 7__. Age category donations 56-65y.

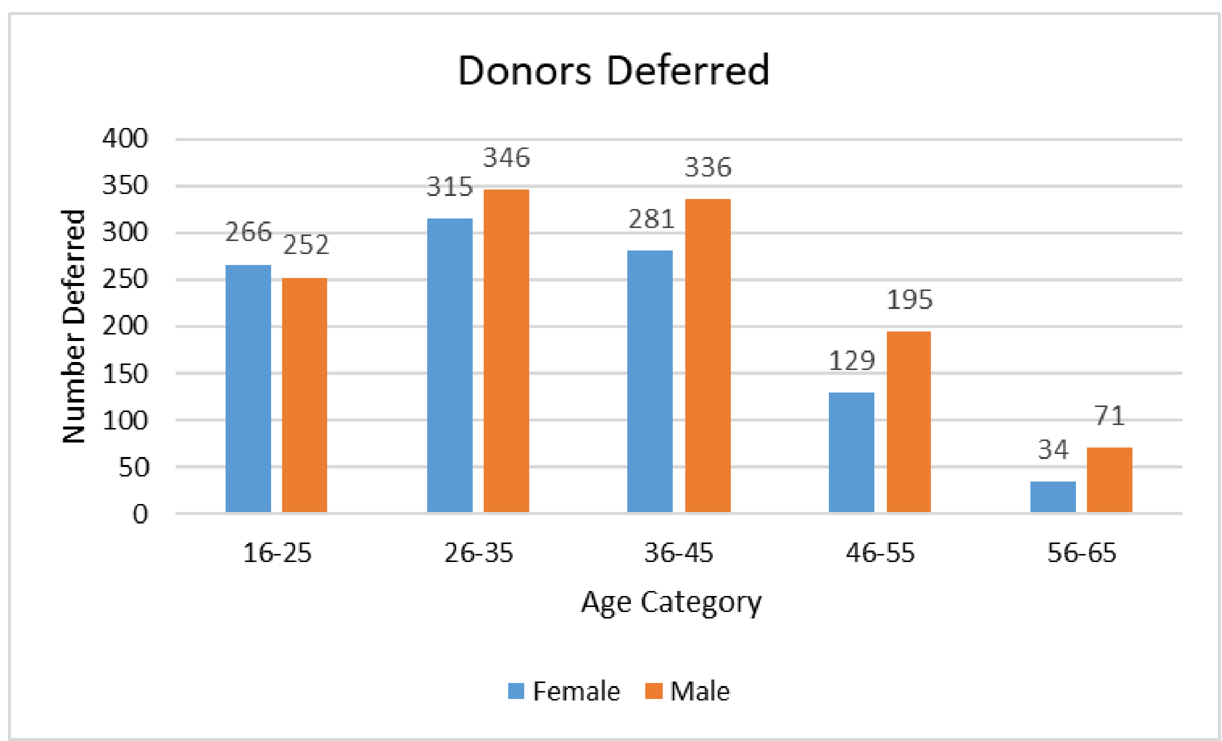


Figure 8. *Donor deferrals during the pandemic (March 2020-March 2022).*

Age and gender deferrals

Figure 8 shows the deferral rates seen in the two genders against different age categories during the pandemic period. It is evident that deferral rates of some age categories varied from others across both the genders during the pandemic.

The two genders show that the age categories with the highest deferral rates were ages between 16-25y, 26-35y, and 36-45y. This is shown to be similar to the age categories with the highest blood donations made during the period, and this indicates a potential correlation between the deferral rates and those willing to donate.

Interview

The interview was conducted with the five members of staff that made up the Department of Blood Services on the impact of COVID-19 on blood donation and the strategy undertaken to recruit donors.

The interview response was coded on common themes and evaluated to show the common response among the interviewees. Fear of infection and restrictions showed a similar cause for lower blood donor turnout. The fear of contacting COIVD during the visit was highlighted in the interview as the individuals were also not aware of the safety measures that were set up for a safer donation (Table 4).

Discussion

The first cases of COVID-19 were reported in March 2020 which led to the major cities and towns of the larger island of Fiji into a lockdown. The cases declined and by December 2020 the nation was on the verge of re-opening to international travel and return to normal day-to-day operations. On April 19th, 2021, the first case of community transmission was announced and a nationwide lockdown was imposed again. During the periods of lockdown, restrictions were in place such as no gathering, maintaining social distances, and the closing of schools and non-essential businesses (Ministry of Health and Medical Services, 2022).

This study aimed at evaluating the impact COVID-19 had on the blood transfusion services by analyzing the supply and demand of blood products during and before the pandemic. The data collected from blood services on blood donations showed a decline each year, as an 8.8% decline was noted between the years 2019 to 2020 and a 4.69% decline between the 2020 and 2021 period. A notable decline in blood donations was captured during the period of lockdown from April 2021 to June 2021, in which blood donations were critically low (Figure 2). A similar result was noted in Maharashtra, India, where blood donor turnout dropped as social distancing and restrictions were imposed. This also led to a decline in blood components (Dhote & Srivastava, 2021). Social restrictions, lockdowns, and the closure of schools, universities, and workplaces had put a halt to blood drives also seen in Hong Kong, which led to a decline in

Table 4. *Perspective of blood service staff on blood donor recruitment during the pandemic.*

Questions	Participants' response	Outcome % (n)
1. What impacts donors from donating during the COVID-19 pandemic?	Fear of infection	100 (n=5/5)
	Lock-down	60 (n=3/5)
	Restrictions	100 (n=5/5)
2. In your opinion, how can donors be recruited for emergency blood needs?	Through donor databases	100 (n=5/5)
	Home to home donor recruitment	40 (n=2/5)
3. In your opinion, what is the suggested solution to overcome the blood shortage?	Awareness	80 (n=4/5)
	Targeting age specific groups	40 (n=2/5)
	Family-based replacement	40 (n=2/5)
4. Is there any strategy in place for recruiting donors in the COVID-19 pandemic?	Hospital staff donations	60 (n=3/5)
	Screening (Temperature check, app etc.)	80 (n=4/5)
	Target non-restricted areas	20 (n=1/5)
5. Are there any changes implemented on the donor criteria form, following the COVID-19 pandemic. If so, what changes have been implemented?	NO	100 (n=5/5)
	COVID-19 vaccination status to be included	60 (n=3/5)

blood donations (Leung & Lee, 2020).

In addition to the lockdown, a major contributing factor was fear as this deterred donors from donating and the majority of donations that came were family-based replacement donors. The other contributing factors were restrictions and lock-down (Table 4). A study by Saud *et al* (2022) on the Saudi population addressed the knowledge and awareness of blood donors on COVID-19 as well as the reasons preventing individuals from donating. The findings showed that most potential donors feared the risk of COVID-19 transmission (Saud *et al* 2021). Similarly, in Greece, following the first cases of COVID-19, a drastic decline in blood supply was noted. This was driven by imposed restrictions and the fear of the unknown (Gkirtsou *et al* 2022).

The ages and genders of donors were evaluated to find the common age category of the blood donor population. The evaluation showed that 86% (n=19210) of the donations were recorded by male donors between January 2019 and March 2022, whereas 14% (n=3112) of the donations were recorded by female donors during the same period (Figure 4).

This finding demonstrated a statistically significant difference in donations by gender distribution (P=0.01826). This could be due to more males presenting for blood donations before and during the pandemic. The results have shown to be common in other studies such as India as male donors tend to show the highest number in total blood donations (Raghuwanshi *et al* 2022).

The higher number of blood donations were the age groups 16-25y, 26-35y and 36-44y when compared to 45-55 and 56-65 years old ($P < 0.00001$) in the male blood donor population. The age category of female donors between 16–25y, 26-35y and 36-44y demonstrated statistical significance (P=0.004416) when compared to the 45–55y and 56-65y age group (Figures 5-7). The same age category showed the highest number of deferrals during the pandemic (Figure 8). A similar result was noted in Iran on the deferral of donors during the COVID-19 pandemic, as the same age category of 25-34y and 35-44y showed the highest rates of deferrals when compared to the other age groups (Mohammed *et al* 2020). A study in the University of Calabar teaching hospital compared donation trends between the COVID

and pre-COVID periods in which they noted that the age category that had the highest number of blood donations was between 15-29y and 30-44y (Ogar *et al* 2021). It can be concluded that this donor population category in Fiji is quite similar to that seen in other countries. The age category between 16y and 25y needs to be targeted as this can help to increase blood donations. This age category also tends to show the least number of deferrals when compared to the number of turnouts in other age categories and with proper education and awareness, donor turnout could increase.

The management of blood component usage was evaluated and a significant reduction in blood component usage was noted during the period of pandemic. An 89% reduction in red cell usage was noted between the years 2019 to 2020 and an 88% was noted between the periods 2020 to 2021. Platelet concentrates showed a 28% decrease in usage between 2019 and 2020. However, there was a slight increase in platelet use of 0.09% between the years 2020 and 2021. Fresh frozen plasma showed a 20% reduced use between the years 2019 to 2020 and an 18% reduced use between 2020 to 2021. Cryoprecipitate showed a 30% reduction in use between 2019 and 2020 and a 19% decrease between 2020 and 2021 (Figure 3). The main reasons for the reduced usage were due to the COVID restrictions in place as there were only admissions of patients with critical conditions or in dire need of a blood transfusion. Surgeries had to be re-scheduled due to the restrictions and availability of blood products. Other contributing factors were hospital outbreaks and staff getting infected with the virus.

The measures taken by countries in studies showed the adoption of policies to reduce blood component usage during the pandemic. Policies implemented to decrease elective surgeries demonstrated reduced blood product usage (Gniadek *et al* 2020). A similar result was noted in a study conducted by Flegel (2020) as blood supply declined. Alternative options such as lowering the number of elective surgeries and component usage have been established through the adoption of new policies. A study conducted at Tan Tock Seng Hospital in Singapore also saw a decrease in blood supply demand during the pandemic as effective measures such as postponing elective surgeries and lowering bed occupancy were undertaken (Fan *et al* 2020). Studies have shown that effective measures taken resulted in a well-managed blood supply level. However, no adoption of policies or strategy was in place during the pandemic at CMWH. Surgeries were rescheduled due to restrictions and a decline in blood stock levels. and blood component usage tends to increase after January 2022, as surgeries were rescheduled, with overseas medical teams conducting major surgeries in the country.

The deferral causes were also evaluated to find the common reasons for deferral during the pandemic in which physical causes showed the highest deferral rate 67% with low haemoglobin and low blood pressure as the commonest causes. During the pandemic, high blood pressure was also seen to increase by 50% from 2020 to 2021. This was also highlighted during the interview of the blood service staff, in which it was noted the blood pressure of the regular donors during the pandemic showed an increase when compared to previous donations before the pandemic. The age category that showed an increase in blood pressure was between 36-45y and 46-55y. This was shown to be common in male donors as a 79% of total high blood pressure deferral rate was recorded between the two genders.

Low haemoglobin deferral was shown to be common in females in 2020, but in 2021 the deferral rate due to low haemoglobin was high in males when compared to females. The deferral trend seen in 2022 followed a similar trend; as of March 2022, high blood pressure alone reached 44% of total deferrals while low haemoglobin had reached 66% of total deferrals when compared to 2021. Low blood pressure deferrals were noted to decrease by 15% between 2020 and 2021.

Through the interview, the perspective of the blood service staff provided an understanding of donor motivation and recruitment during the pandemic. It was identified that donors were recruited through donor databases (100%) and home-to-home donations (40%) during the pandemic. The blood service staff raised issues that there was a lack of awareness (100%) of the public on the safety measures in place for a safe donation with minimal risk of infection and protocols on social distancing maintained. A similar scenario was seen when a survey was conducted in seven European countries (Denmark, Germany, France, UK, Italy, Portugal, and Netherlands) by Torsten *et al* (2021) to understand the motivation of blood donors during the pandemic. The results showed a decline in the donation trend, in which the researchers noted that the cause was this lack of awareness (Torsten *et al* 2021).

The approach undertaken by the blood services at CWMH was to defer donors during the pandemic on temperature screening and symptoms that were related to COVID-19. Other implementations were to target non-restricted areas for donor recruitment. In the pandemic, the hospital underwent a lockdown due to an outbreak of COVID-19 amongst hospital staff. A suggested measure was to take blood donations from staff that were within the premises. This would have been through a set of screening measures along with the donor criteria adopted during the pandemic to identify potential donors.

Through the interview it was noted that there were no changes made to the donor criteria form following the pandemic. It was highlighted that there are suggestions for looking into changing some of the donor recruiting criteria. A major setback in the criteria form was recruiting donors after vaccination, as it was still unclear when donors could donate after getting their vaccination dose. A well-structured form can therefore help identify potential donors during times of pandemics or major outbreaks.

Limitations

There was no data available on donors recruited for the month of May and August at blood services due to the data being lost during transition from tent (out-center) screening to in-center. The data from the blood bank was utilized on the number of donors screened during these months, this limited the study as there was no age and gender information captured.

Conclusions

The world underwent a devastating pandemic that caused economic and social disruption. The health sector was one of the major sectors affected as supplies were running low and the number of patients hospitalized was increasing daily. The blood service department at CMWH faced a similar situation as seen around the world; blood stock levels drastically declined with a lower donor turnout. The fear of the unknown deterred people from coming forward to donate. Other imposed restrictions made it difficult for those who were willing to donate. Blood donations were solely relied on by family replacement, and most family members were hesitant to come out during the pandemic.

Blood component usage rates declined with the pandemic as major surgeries were rescheduled; however, no policies were in place that controlled the regulation of blood components during the pandemic. Adoption of policies have been evident in other studies to manage blood products during the pandemic and a structured system can be beneficent not just for COVID but other similar scenarios the country undergo like a measles outbreak and natural disasters like cyclones.

Donor deferral rates saw a drastic incline as the community outbreak increased. The common causes of deferral were low haemoglobin and low or high blood pressure. The age categories of 16-25y ,26 35y and 36-45y showed the highest numbers of donations as well as deferral rates. Other age categories like 16–25y can be targeted in getting potential donors. This can be achieved through proper education and planning.

The study has highlighted the major impacts of COVID on blood transfusion services at CWMH in which a strategic approach needs to be adopted for scenarios such as the pandemic, as studies have shown that the adoption of policies has worked in combating the pandemic regarding blood supply and demand.

Recommendations

A strategy or a policy should be in place for the regulation of blood components and elective surgeries during the pandemic or in other such scenarios. The adoption of guidelines has shown to work during these times, as studies have revealed that the approaches taken have been effective in showing a well-managed blood stock.

Blood donor recruitment guidelines need to be established. These guidelines can help in identifying potential donors as well as provide safety to the blood service staff and make the recruitment process less stringent. The process of donor recruitment was quite a challenge as the protocols were not documented.

Blood donations needs to be targeted with age categories as the donor age criteria has changed from 18-60y to 16y to 65y. The age category 16y to 25y needs to be targeted as a significant rise was shown as the change in donor age criteria was implemented. This can be achieved by targeting secondary schools and university students, with proper awareness and information provided about blood donation.

Common causes of deferral such as low haemoglobin and high blood pressure needs to be addressed while recruiting donors; this could be by recommendations made on their diet or lifestyle. This could not only be as a recommendation but also as a motivation for donors to return for donation after making these changes with a follow-up sent through email or messages to the deferred donors.

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References

Almalki S, Asseri M, Khawaji Y, Alqurashi R, Badawi M, Yakout N, Elgemmezi T, Hindawi S 2021. Awareness about Coronavirus (COVID-19) and challenges for blood services among potential blood donors. *Transfusion and Apheresis Science, 60*(6), p.103211.

Al-Riyami AZ, Abdella YE, Badawi MA, Panchatcharam SM, Ghaleb Y, Maghsudlu M, Satti M, Lahjouji K, Merenkov Z, Adwan A, Feghali R 2021. The impact of COVID-19 pandemic on blood supplies and transfusion services in Eastern Mediterranean Region. *Transfusion Clinique et Biologique, 28*(1), pp.16-24.

Biswas D, Maiti C, Talukder B, Azharuddin M, Saha S, Pandey S, Das A, Adhikari SD, Ray Y, Sarkar BS, Paul SR 2021. A prospective study on COVID-19 convalescent plasma donor (CCP) recruitment strategies in a resource constrained blood centre. *ISBT Science Series, 16*(4), pp.276-283.

Bouhou S, Benajiba M 2020. Ensuring a safe and adequate blood supply during the COVID-19 pandemic: the Moroccan National Blood Center experience. *Pan African Medical Journal, 37*(1).

Cai X, Ren M, Chen F, Li L, Lei H, Wang X 2020. Blood transfusion during the COVID-19 outbreak. *Blood Transfusion, 18*(2), p.79.

Chandler T, Neumann-Böhme S, Sabat I, Barros PP, Brouwer W, Van Exel J, Schreyögg J, Torbica A, Stargardt T 2021. Blood donation in times of crisis: early insight into the impact of COVID-19 on blood donors and their motivation to donate across European countries. *Vox Sanguinis, 116*(10), pp.1031-1041.

Chen S, Yang J, Yang W, Wang C, Bärnighausen T 2020. COVID-19 control in China during mass population movements at New Year. *The Lancet, 395*(10226), pp.764-766.

Chiem C, Alghamdi K, Nguyen T, Han JH, Huo H, Jackson D 2022. The impact of COVID-19 on blood transfusion services: a systematic review and meta-analysis. *Transfusion Medicine and Hemotherapy, 49*(2), pp.107-118.

Choudhury N, Mathur A, Smit Sibinga CT, On behalf of AATM 2020. COVID-19 Pandemic–blood supply challenges and approaches in AATM member countries. *ISBT Science Series, 15*(4), pp.353-361.

Critical blood shortage in Fiji. RNZ. (2022). Retrieved 29 March 2022, from https://www.rnz.co.nz/international/pacific-news/448904/critical-blood-shortage-in-fiji.

Delabranche X, Kientz D, Tacquard C, Bertrand F, Roche AC, Tran Ba Loc P, Humbrecht C, Sirlin F, Pivot X, Collange O, Levy F, 2021. Impact of COVID-19 and lockdown regarding blood transfusion. *Transfusion, 61*(8), pp.2327-2335.

DeSimone RA, Costa VA, Kane K, Sepulveda JL, Ellsworth GB, Gulick RM, Zucker J, Sobieszczyk ME, Schwartz J, Cushing MM 2021. Blood component utilization in COVID-19 patients in New York City: transfusions do not follow the curve. *Transfusion, 61*(3), pp.692-698.

Dhiman Y, Patidar GK, Arora S 2020. Covid-19 pandemic-response to challenges by blood transfusion services in India: a review report. *ISBT Science series, 15*(4), pp.365-373.

Dhote SW, Srivastava AR 2021. Trends of Blood Transfusion Services before and during COVID-19 Pandemic-A Retrospective Study from Maharashtra, India. *Journal of Clinical & Diagnostic Research, 15*(12).

Elsafi SH 2020. Demographical pattern of blood donors and pre-donation deferral causes in Dhahran, Saudi Arabia. *Journal of Blood Medicine*, pp.243-249.

Fan BE, Ong KH, Chan SSW, Young BE, Chong VCL, Chen SPC, Lim SP, Lim GP, Kuperan P 2020. Blood and blood product use during COVID-19 infection. *American Journal of Hematology, 95*(7), p.E158.

Flegel WA 2020. CoVID-19 insights from transfusion medicine. *British Journal of Haematology, 190*(5), p.715.

García-Erce JA, Romón-Alonso Í, Jericó C, Domingo-Morera JM, Arroyo-Rodríguez JL, Sola-Lapeña C, Bueno-Cabrera JL, Juárez-Vela R, Zalba-Marcos S, Abad-Motos A, Gea-Caballero V 2021. Blood donations and transfusions during the COVID-19 pandemic in Spain: impact according to autonomous communities and hospitals. *International Journal of Environmental Research and Public Health, 18*(7), p.3480.

Gehrie E, Tormey CA, Sanford KW 2020. Transfusion service response to the COVID-19 pandemic. *American Journal of Clinical Pathology, 154*(3), pp.280-285.

Gkirtsou C, Konstantinidis T, Cassimos D, Konstantinidou EI, Kontekaki EG, Rekari V, Bezirtzoglou E, Martinis G, Stergiannis P, Thrace Study Group on Blood Donors' Attitude during the COVID-19 Pandemic 2022. Views and attitudes of blood donors toward blood donation during the COVID-19 pandemic in Thrace Region, Greece. *International Journal of Environmental Research and Public Health, 19*(9), p.4963.

Gniadek TJ, Mallek J, Wright G, Saporito C, AbiMansour N, Tangazi W, Rogers G, Zahara Z, Cummings G, Kaul K, Kang J 2020. Expansion of hospital-based blood collections in the face of COVID-19 associated national blood shortage. *Transfusion, 60*(7), pp.1470-1475.

Grandone E, Mastroianno M, Caroli A, Ostuni A 2020. Blood supply and transfusion support in southern Italy: findings during the first four weeks of the SARS-CoV-2 pandemic. *Blood Transfusion, 18*(3), p.230.

Hakami NY, Al-Sulami AJ, Alhazmi WA, Qadah TH, Bawazir WM, Hamadi AY, Owaidah AY, Alhefzi RA, Hamdi FY, Maqnas A, Alghassab GF 2022. Impact of COVID-19 on blood donation and supply: a multicenter cross-sectional study from Saudi Arabia. *BioMed Research International, 2022*.

Kandasamy D, Shastry S, Chenna D, Mohan G 2022. COVID-19 pandemic and blood transfusion services: The impact, response and preparedness experience of a tertiary care blood center in southern Karnataka, India. *Hematology, Transfusion and Cell Therapy, 44*, pp.17-25.

Leung JN, Lee CK 2020. Impact of the COVID-19–a regional blood centre's perspective. *ISBT Science Series, 15*(4), pp.362-364.

Li Z, Lei S, Li X, Zhao Y, Dai Y, Jin S, Fu Q, Cai X, Lin Z, Tu X 2021. Blood donation fear, perceived rewards, self-efficacy, and intention to return among whole blood donors in China: A social cognitive perspective. *Frontiers in Psychology, 12*, p.683709.

Loua A, Kasilo OMJ, Nikiema JB, Sougou AS, Kniazkov S, Annan EA 2021. Impact of the COVID-19 pandemic on blood supply and demand in the WHO African region. *Vox Sanguinis, 116*(7), pp.774-784.

Lu W, Yazer M, Li N, Ziman A, Wendel S, Tang H, Tsang H, Titlestad K, Thibodeaux SR, Shih AW, Poisson JL 2022. Hospital red blood cell and platelet supply and utilization from March to December of the first year of the COVID-19 pandemic: The BEST collaborative study. *Transfusion, 62*(8), pp.1559-1570.

Masser BM, Hyde MK, Ferguson E 2020. Exploring predictors of Australian community members' blood donation intentions and blood donation–related behavior during the COVID-19 pandemic. *Transfusion, 60*(12), pp.2907-2917.

Ministry of Health & Medical Services 2022. Retrieved 29 March 2022, from https://www.health.gov.fj/.

Miskeen E, Omer Yahia AI, Eljack TB, Karar HK 2021. The impact of COVID-19 pandemic on blood transfusion services: a perspective from health professionals and donors. *Journal of Multidisciplinary Healthcare*, pp.3063-3071.

Mohammadi S, Yazdi SMT, Eshghi P, Norooznezhad AH 2020. Coronavirus disease 2019 (COVID-19) and decrease in blood donation: experience of Iranian Blood Transfusion Organization (IBTO). *Vox Sanguinis, 115*(7), p.595.

Ngo A, Masel D, Cahill C, Blumberg N, Refaai MA 2020. Blood banking and transfusion medicine challenges during the COVID-19 pandemic. *Clinics in Laboratory Medicine, 40*(4), pp.587-601.

Noordin SS, Yusoff NM, Karim FA, Chong SE 2021. Blood transfusion services amidst the COVID-19 pandemic. *Journal of Global Health, 11*.

Ogar CO, Okoroiwu HU, Obeagu EI, Etura JE, Abunimye DA 2021. Assessment of blood supply and usage pre-and during COVID-19 pandemic: a lesson from non-voluntary donation. *Transfusion Clinique et Biologique, 28*(1), pp.68-72.

Ondrasik RM, Khan J, Szczepiorkowski ZM, Levy JJ, Dunbar NM 2022. Passive order auditing associated with reductions in red blood cell utilization: National blood shortage experience. *Transfusion, 62*(8), pp.1551-1558.

Ou-Yang J, Li SJ, Bei CH, He B, Chen JY, Liang HQ, Fu YS 2020. Blood donor recruitment in Guangzhou, China, during the 2019 novel coronavirus (COVID-19) epidemic. *Transfusion, 60*(11), pp.2597-2610.

Pagano MB, Hess JR, Tsang HC, Staley E, Gernsheimer T, Sen N, Clark C, Nester T, Bailey C, Alcorn K 2020. Prepare to adapt: blood supply and transfusion support during the first 2 weeks of the 2019 novel coronavirus (COVID-19) pandemic affecting Washington State. *Transfusion, 60*(5), pp.908-911.

Pagano MB, Rajbhandary S, Nunes E, Cohn CS 2020. Transfusion services operations during the COVID-19 pandemic: Results from AABB survey. *Transfusion, 60*(11), p.2760.

Pandey HC, Coshic P, CS C, Arcot PJ, Kumar K 2021. Blood supply management in times of SARS-CoV-2 pandemic–challenges, strategies adopted, and the lessons learned from the experience of a hospital-based blood centre. *Vox Sanguinis, 116*(5), pp.497-503.

Quaglietta A, Nicolucci A, Posata R, Frattari A, Parruti G, Accorsi P 2021. Impact of Covid-19 epidemic on the activities of a blood centre, transfusion support for infected patients and clinical outcomes. *Transfusion Medicine, 31*(3), pp.160-166.

Rafiee MH, Kafiabad SA, Maghsudlu M 2021. Analysis of blood donors' characteristics and deferrals related to COVID-19 in Iran. *Transfusion and Apheresis Science, 60*(2), p.103049.

Raghuwanshi B, Behera P, Singh P, Khan R, Munshi R, Patil A, Chouhan S 2022. Blood supply management amid COVID 19 pandemic: Challenges and strategies. *Journal of Family Medicine and Primary Care, 11*(6), p.2363.

Routray SS, Ray GK, Prakash S, Sahu A, Naik A, Mukherjee S 2022. Impact of COVID-19 on blood donor deferral patterns during the COVID-19 pandemic: A retrospective analysis. *Vox Sanguinis, 117*(5), pp.656-663.

Sachdev S, Kishore K, Singh L, Lamba DS, Hans R, Dhawan HK, Grover S, Sharma RR 2021. Exploration of COVID-19 related fears deterring from blood donation in India. *ISBT Science Series, 16*(2), pp.147-157.

Schiroli D, Merolle L, Molinari G, Di Bartolomeo E, Seligardi D, Canovi L, Pertinhez TA, Mancuso P, Giorgi Rossi P, Baricchi R, Marraccini C 2022. The impact of COVID-19 outbreak on the Transfusion Medicine Unit of a Northern Italy Hospital and Cancer Centre. *Vox Sanguinis, 117*(2), pp.235-242.

Shander A, Goobie SM, Warner MA, Aapro M, Bisbe E, Perez-Calatayud AA, Callum J, Cushing MM, Dyer WB, Erhard J, Faraoni D 2020. Essential role of patient blood management in a pandemic: a call for action. *Anesthesia and Analgesia*.

Silva-Malta MCF, Rodrigues DDOW, Chaves DG, Magalhães NNS, Ribeiro MA, Cioffi JGM, Martins, ML 2021. Impact of COVID-19 in the attendance of blood donors and production on a Brazilian Blood Centres. *Transfusion Medicine, 31*(3), pp.206-212.

Stanworth SJ, New HV, Apelseth TO, Brunskill S, Cardigan R, Doree C, Germain M, Goldman M, Massey E, Prati D, Shehata N 2020. Effects of the COVID-19 pandemic on supply and use of blood for transfusion. *The Lancet Haematology, 7*(10), pp.e756-e764.

Tagny CT, Lendem I, Ngo Sack F, Ngo Balogog P, Ninmou C, Dongmo A, Ndoumban A, Tante E, Ateba N, Ndemanou M, Tapko JB 2021. Trends in blood donations, blood donors' knowledge, practices and expectations during the COVID-19 pandemic in Cameroon. *Vox Sanguinis, 116*(6), pp.637-64.

Turner TR, Olafson C, Mykhailova O, Xu A, Acker JP 2020. Evaluating blood product quality post expiry to mitigate blood shortages during the COVID-19 pandemic in Canada. *Transfusion, 60*(12), p.3072.

Velázquez-Kennedy K, Luna A, Sánchez-Tornero A, Jiménez-Chillón C, Jiménez-Martín A, Vallés Carboneras A, Tenorio M, García García I, López-Jiménez FJ, Moreno-Jiménez G 2021. Transfusion support in COVID-19 patients: impact on hospital blood component supply during the outbreak. *Transfusion, 61*(2), pp.361-367.

Wang Y, Han W, Pan L, Wang C, Liu Y, Hu W, Zhou H, Zheng X 2020. Impact of COVID-19 on blood centres in Zhejiang province China. *Vox Sanguinis, 115*(6), pp.502-506.

Warner MA, Patel PA, Hensley NB, Mazzeffi M 2022. COVID-19-Related Blood Shortages and Cardiac Surgery: Do We Have Too Many Eggs in One Basket?. *Journal of Cardiothoracic and Vascular Anesthesia, 36*(7), pp.1823-1826.

Warner MA, Patel PA, Hensley NB, Mazzeffi M 2022. COVID-19-Related Blood Shortages and Cardiac Surgery: Do We Have Too Many Eggs in One Basket?. *Journal of Cardiothoracic and Vascular Anesthesia, 36*(7), pp.1823-1826.

Yahia AIO 2020. Management of blood supply and demand during the COVID-19 pandemic in King Abdullah Hospital, Bisha, Saudi Arabia. *Transfusion and Apheresis Science, 59*(5), p.102836.

Yuan Z, Chen D, Chen X, Wei Y 2020. Estimation of the number of blood donors during the COVID-19 incubation period across China and analysis of prevention and control measures for blood transfusiontransmission. *Tranfusion, 60*(8), pp.1778-1784.

ORIGINAL ARTICLE

Strategies for isolating microbiota from faecal samples: are all gut microorganisms potentially cultivable?

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Abstract

The gut microbiome is complex and diverse and consists of bacteria that can be difficult to grow due to challenges in mimicking the natural gut environment in vitro. While molecular techniques have been used to identify gut bacteria, the culture and isolation of gut bacteria in the laboratory is still considered an essential step for the interpretation of metagenomic data and to gain a better understanding of the role of gut microbiota in human health.

There have been significant advances in the cultivation of gut bacteria in recent years however a large percentage of gut bacteria, particularly anaerobes, remain difficult to culture. This systematic review was undertaken to determine what culture methods have been used to isolate and identify various gut bacteria from human stool samples. Novel approaches that have been developed to overcome some of the challenges associated with traditional culture-based methods are also discussed.

Keywords: ***gut microbiota, anaerobes, gut bacteria, microbiome, bacterial culture***

Introduction

The gut microbiota

The human gastro-intestinal (GI) tract harbors approximately 10^{12} microorganisms, mostly bacteria but also viruses, fungi, and protozoa. The number of bacteria in various parts of the intestine can vary with the highest number found in the large intestine. The total number of microbiota are thought to outnumber host cells by at least a factor of 10 (Conlon and Bird 2014). It is therefore not surprising that the three million genes plus encoded by microorganisms compared to the human genome of about 23,000 genes, play a crucial role in host physiology and health (Valdes *et al* 2018; Belkaid and Hand 2014). The GI tract is also the largest compartment within the human body where host tissues interact with the external environment (Alkanani *et al* 2015).

Molecular techniques have revealed that 90% of the gut microbiota is composed of four major microbial phyla: Firmicutes, Bacteroides, Proteobacteria and Actinobacteria (Bibbo *et al* 2017), with the most prevalent genera of bacteria in the human gut being *Bifidobacterium* spp., *Lactobacillus* spp., *Bacteroides* spp., *Clostridium* spp., *Escherichia* spp., *Streptococcus* spp. and *Ruminococcus* spp. (Conlon and Bird 2014). The beneficial contribution of gut microbiota is well recognised and contributes to faecal bulk and metabolic functions, especially fermentation and absorption of undigested carbohydrate, and produces physiologically important products such as short chain fatty acids (SCFA), vitamins and lipids (Conlon and Bird 2014). However, some end products of protein metabolism such as ammonia, phenols and hydrogen sulphide can be toxic (Conlon and Bird 2014).

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There is growing evidence suggesting that the interaction between the environment, the gut microbiome and the host can contribute to the development of several diseases, such as non-alcoholic steatohepatitis(Rojo *et al* 2017). In an optimal immune system–microbiota partnership there is a symbiotic relationship between the two and the exposure to microorganisms helps in the development of an effective immune system, which adapts to provide protective responses to pathogens and tolerance of innocuous antigens (Belkaid and Hand 2014). The gut microbiota protects against the over-growth of pathogenic organisms and helps maintain the integrity of the intestinal wall and contributes to metabolic functions (Alkanani *et al* 2015). Several studies found an altered composition of the gut microbiota is

associated with the development and progression of disorders including diabetes (making the gut microbiota an important area for research over the last few decades (Bibbo *et al* 2017; Pereira and Cunha 2020).

Culture-dependent methods in gut microbiota research

Bacterial culture methods, first developed by Louis Pasteur and Robert Koch from the mid-1800s to early 1900s, represent the initial methods used to study microorganisms (Bonnet *et al* 2020). The development of anaerobic cultivation techniques in the 1960s and 1970s was a major breakthrough in the study of gut microbiota allowing the cultivation of many species of fastidious intestinal bacteria that microbiologists had not been able to isolate earlier (Lagkouvardos *et al* 2017).

Research advanced dramatically with the advent of sequencing technologies, expanding our knowledge of the diversity of the human gut microbiota (Lagier *et al* 2012). The discovery of new species and strains with molecular-based methods highlighted that a large number of gut microbiota are not readily isolated with traditional culture methods (Sommer 2015). The gut microbiota is complex and diverse (Chen *et al* 2019) and consists mostly of anaerobic bacteria (Rettedal *et al* 2014). It also has other characteristics, such as the requirement for factors produced by other microbes, interspecies competition/inhibition, dormancy and slow growth. Some of these characteristics have been associated with the challenges in growing some gut bacteria (Cross *et al* 2019). Following years of focussing on molecular methods for the identification of microorganisms that have been difficult to isolate using culture techniques, cultivation methods have more recently been considered a key step to gain a full understanding of the role of human gut bacteria in health and disease (Chang *et al* 2019; Goldman *et al* 2022).

More recent approaches such as culturomics, have been used by scientists to grow several species of gut bacteria that were not previously detected by molecular techniques (Sommer 2015). In the culturomics approach, microorganisms from clinical specimens such as faeces are grown using a wide range of media and growth temperatures, increasing the chances of growing fastidious bacteria as various bacteria may grow under different conditions. This approach can be time consuming as it requires bacteria to be grown in media under a range of conditions some of which may include extended incubation times. High-throughput identification methods such as MALDI-TOF MS mass spectrometry and 16S rRNA gene sequencing are used to rapidly identify any organisms that are cultured by this technique (Lagier *et al* 2015; Lagier *et al* 2018). More recently an automated culturomics method that involves the use of robotics to study colony morphology and genomics has been described and this may reduce the time taken to isolate and identify organisms making this method more attractive for the identification of novel gut microbes (Huang *et al* 2023).

With the growing appreciation of the importance of isolating intestinal bacteria, there has been a major push to improve standard culturing approaches and develop innovative culturing methods to isolate members of yet uncultivated gut bacteria and has become a major field of interest (Chang *et al* 2019).

This paper aims to review studies that have been published on methods used to culture human gut microbiota, focusing on anaerobes which are generally difficult to isolate and grow in the laboratory.

Method

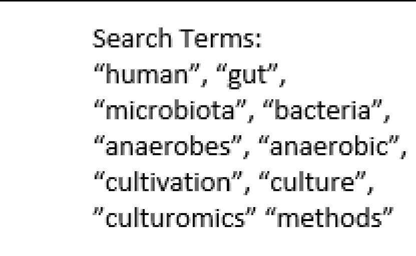
This systematic review was performed according to the PRISMA standard guidelines for meta-analyses and systematic reviews (Moher *et al* 2008). A literature search and review of published articles was conducted to find relevant papers for this review. The search was conducted on PubMed and databases accessed via WorldWideScience. The search terms used were “human”, “gut”, “microbiota”, “bacteria”, “anaerobes”, “anaerobic”, “cultivation”, “culture”,” culturomics”, “methods”. The search was limited to articles published between 2010 and 2022. From the search results, the titles, abstracts, and methods of published papers were examined. Once duplicates had been eliminated, 920 papers were identified using these search terms. Of these, only primary literature relating to original research studies using culture-dependent methods to grow and isolate human gut microbiota and those that included details on the media used were included in the review. This led to a total of 66 papers being identified for inclusion in this review. The process of selecting papers for this review is described in Table 1.

Results and Discussion

After reviewing the literature, it was found that a range of media with various culture conditions was used with variable success. Identification of bacteria isolated using culture methods predominantly involved molecular methods such as sequencing the 16s rRNA gene (Table 2).

The papers were underpinned by the theory that all microorganisms are potentially “cultivable” with the appropriate conditions to isolate them. Two early pioneering studies (Goodman *et al* 2011;

Table 1. Process of identifying and selecting suitable papers to include in the review. Various search terms as shown were entered into databases such as PubMed and WorldWideScience in order to identify suitable publications. Papers were excluded when they did not include primary data and/or did not include details regarding culture techniques and information on media used to grow microorganisms.



Records identified through database searching: PubMed (116), WorldWideScience (436), ASM Journals (369) (n=921)

Additional records identified through other sources (e.g. Google Scholar)

Records after duplicates removed (n=920)

Records screened (n=561)

Records excluded (n=469)

Full-text articles assessed for eligibility (n=92)

Full-text articles excluded, (n=26)

Studies included in qualitative synthesis (n=66)

Lagier *et al* 2012), support this and demonstrate that novel gut bacteria could be isolated with extensive culturing approaches.

Commonly used media

While many types of media were used across the studies, it was apparent that some media were more commonly used than others and these include Brain Heart Infusion Agar, Columbia Agar, Gifu Anaerobic media, Gut Microbiota Medium, MacConkey Agar and Tryptic Soy Agar (Figure 1). Further details on media and culture conditions described in the papers are listed in Table 2. Most of these are media that are relatively affordable and easy to access in any microbiology laboratory. They were often modified though to suit growth requirements of various bacteria. This suggests that while researchers were able to use commonly available media that would usually be used to cultivate a range of bacteria, the successful isolation of difficult to isolate gut bacteria was dependent upon the use of modifications including the addition of specific components such as antibiotics or 5% sheep blood well.

Some studies have focussed on extended periods of enrichment as seen in Chang *et al* (2019) who performed a 30-day continuous enrichment in blood culture bottles in their study and found that having an extended enrichment step ensured a better isolation of different bacterial species when fresh medium was added to the enriched culture (Chang *et al* 2019). It was clear that many researchers went to significant lengths to test various growth conditions before they were finally successful. This highlights the single biggest disadvantage to bacterial culture which is the length of time required to obtain a pure culture.

The most widely used growth temperature was 37°C and anaerobic growth conditions were used as the aim was to isolate anaerobes. The addition of CO2 to the incubation atmosphere was described in some studies; either 5% (Lau *et al* 2016) or 2.5% (Dubourg *et al* 2013).

In most cases incubation periods ranged from 48hrs to up to a week. Less commonly incubation periods of a month or more were reported (Caputo *et al* 2015; Dubourg *et al* 2013). Identification of isolates was mostly by 16S rRNA gene sequencing followed by MALDI-TOF. The list of all media reported in studies reviewed in this paper are listed in Table 2.

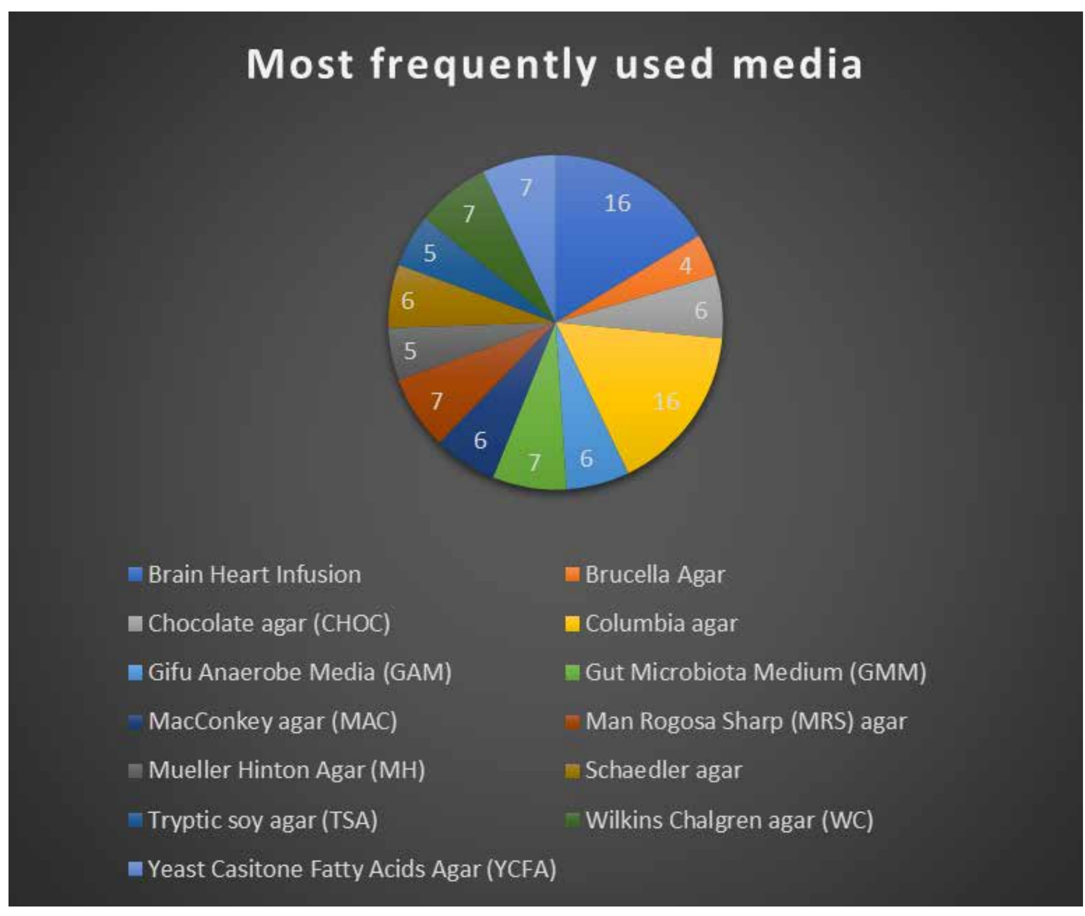


***Figure 1**. Chart of the most commonly used media for culturing the microbiota samples.*

Table 2*. Culturing media and conditions for isolation of faecal bacteria from human stool samples[1].*

Culture Medium	Pre-incubation[1]	Ethanol pre-treatment	Addition of antibiotics	Filtration	Culturing conditions (Atmosphere, incubation temperature and incubation time)	Identification methods and related information	Reference
Actinomyces Isolation Agar (AIA)					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (AGAGTTTGATCCTGGCTCAG) and 927r (CCGTCAATTC CTTTRAGTTT)	(Lau *et al* 2016)
Agar-Agar Medium					Anaerobic atmosphere Incubation temperature and time: not available	16S rRNA gene sequencing	(Zou *et al* 2019)
All Culture Agar (AC)					Anaerobic atmosphere Incubation temperature and time: not available	16S rRNA gene sequencing	(Zou *et al* 2019)
Anaerobe Agar					Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks, and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
AT (Peptone, Beef Extract, NaCl) Medium					Anaerobic atmosphere Incubation temperature and time: Not available	16S rRNA gene sequencing	(Zou *et al* 2019)
Bacteroides Bile Esculin Agar (BBE)					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (CCTACGGGAGGCAGCAG-) and 926R (CCGTCAATTCCTTTRAGTTT)	(Ito *et al* 2019)
					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing	(Lau *et al* 2016)
Baird Parker Agar					Not available	16S rRNA gene sequencing	(Carroccio *et al* 2021)

Beef and Liver Agar + 5% Sheep Blood + Metronidazole 100 µg + Vancomycin 100 µg			✓		Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Bifidobacterium Selective Media (BSM)					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (AGAGTTTGATCCTGGCTCAG-3') and 927r (CCGTCAATTC CTTTRAGTTT)	(Lau *et al* 2015)
			✓		Anaerobic atmosphere Incubation temperature: not available 2-3 days	Statistical anaylyses of bacterial counts	(Mikkelsen *et al* 2015)
					Anaerobic atmosphere 37°C 72 hours	Bacteria morphology, Gram staining, catalase and oxidase tests, and growth under selective conditions	(Drago *et al* 2012)
Bifidobacterium Selective Media (BSM), modified					Not available	16S rRNA gene sequencing	(Carroccio *et al* 2021)
Bordetella Agar					Aerobic atmosphere with 2.5% $C0_2$ 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Aerobe atmosphere with 2.5% $C0_2$ 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Brain Heart Infusion Agar (BHI)					Aerobic atmosphere 37°C 24 hours Anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Region: V4 Primers/Sequences: 515F/806R (TCCTACGGGAGGCAG CAGT/ GGACTACCAGGGTATCTAATCCTGTT)	(Cha *et al* 2018)

			✓	✓	Anaerobic atmosphere 55°C Incubation time: not available Aerobic atmosphere 57°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Aerobic and anaerobic atmosphere 37°C 72 hours	16S RNA gene sequencing Complete 16S RNA gene sequencing by Sanger method	(Torres-Sanchez *et al* 2021)
					Aerobic atmosphere 57°C 2 days, 1 week, 2 weeks, and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
					Anaerobic atmosphere Incubation temperature and time: not available	16S RNA gene sequencing	(Zou *et al* 2019)
					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (CCTACGGGAGGCAGCAG-) and 926R (CCGTCAATTCCTTTRAGTTT)	(Ito *et al* 2019)
		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)
					Aerobic and anaerobic atmosphere 37°C 72 hours	Whole Genome Sequencing (WGS), phylogenomic identification, and specific gene-encoding searches	(López-Moreno *et al* 2022)
					Aerobic atmosphere 55°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (GAATTGACGGGGRCCC) and 1391R (GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)[2]
	✓				Anaerobic atmosphere 37°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (GAATTGACGGGGRCCC) and 1391R (GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)

					Anaerobic atmosphere 55°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS 16S rRNA gene sequencing Clone sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
	✓				Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (AGAGTTTGATCCTGGCTCAG) and 927r (CCGTCAATTC CTTTRAGTTT)	(Lau *et al* 2016)
	✓				Anaerobic atmosphere 37°C 24 to 72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
		✓			Anaerobic atmosphere 37°C 24 to 72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
Brain Heart Infusion (BHI) + NaCl 1 g/l					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
Brain Heart Infusion (BHI) + NaCl 3 g/l					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Aerobic atmosphere 37°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (GAATTGACGGGGRCCC) and 1391R (GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)
Brain Heart Infusion (BHI) 0.8 µm Filtration				✓	Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)

Brain Heart Infusion (BHI) 5 µm Filtration				✓	Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Brain Heart Infusion (BHI) + NaCl 15 g/L					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Brain Heart Infusion (BHI) + NaCl 1 g/L					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Brain Heart Infusion (BHI) + NaCl 3 g/L					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Brain Heart Infusion (BHI) + 5% Sheep Blood					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
Brain Heart Infusion (BHI) + 5% Sheep Blood					Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Brain Heart Infusion (BHI) + 5% Sheep Blood + Metronidazole 100 µg + Vancomycin 100 µg			✓		Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)

Brain Heart Infusion (BHI) + Vancomycin			✓		Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Brain Heart Infusion (BHI)				✓	Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Brain Heart Infusion (BHI) Supplemented with 5 g/L Yeast Extract + 1 g/L Cysteine + 15 mg/L Hemin (only in agar) (BHIych)					Anaerobic atmosphere 37°C 3-7 days	16S rRNA gene sequencing primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) were used to amplify part of the 16S rRNA gene	(Fenn *et al* 2017)
Brain Heart Infusion (BHI) Supplemented with 5 g/L Yeast Extract + 0.5 g/L Cysteine + 5 mg/L Hemin + 1 g/L Cellobiose + 1 g/L Maltose (LYHBHI)					Anaerobic atmosphere 37°C 3-7 days	16S rRNA gene sequencing primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) were used to amplify part of the 16S rRNA gene	(Fenn *et al* 2017)
Brain Heart Infusion (BHI), NaCl 3 g/L					Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Brain Heart Infusion (BHI) + Cellulose					Anaerobic atmosphere 37°C 5 days	16S rRNA gene sequencing Regions: V4-V5	(Goldman *et al* 2022)
Brain Heart Infusion (BHI) + Pectin					Anaerobic atmosphere 37°C 5 days	16S rRNA gene sequencing Regions: V4-V5	(Goldman *et al* 2022)
Brain Heart Infusion (BHI) + Starch					Anaerobic atmosphere 37°C 5 days	16S rRNA gene sequencing Regions: V4-V5	(Goldman *et al* 2022)

Brain Heart Infusion (BHI) + 5% Sheep Blood					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Bromocresol Purple Lactose Agar (BCP)					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Anaerobic atmosphere 37°C 48 hours	MALDI-TOF MS 16S rRNA gene sequence Regions: V4-V5	(Samb-Ba *et al* 2014)
					Aerobic atmosphere 37°C 48 hours	MALDI-TOF MS 16S rRNA gene sequence Regions: V4-V5	(Samb-Ba *et al* 2014)
Brucella Agar					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Aerobic atmosphere 37 °C 1 day, 1 week, 2 week and 1 month	MALDI-TOF MS	(Pfleiderer *et al* 2013)
		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)
					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)

Buffered Charcoal Yeast Extract (BCYE)					Aerobic atmosphere with 2.5% CO_2 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
					Aerobic atmosphere with 2.5% CO_2 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
Buffered CYE Differential Agar					Anaerobic atmosphere 37°C 48 hours	MALDI-TOF MS 16S rRNA gene sequence Region: V4-V5	(Samb-Ba *et al* 2014)
					Aerobic atmosphere 37°C 48 hours	MALDI-TOF MS 16S rRNA gene sequence Regions: V4-V5	(Samb-Ba *et al* 2014)
CaCO3 Agar					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Campylobacter Blood Agar (CBA)					Microaerophilic atmosphere 42°C 48 hours	MALDI-TOF MS	(He *et al* 2010)
Cefsulodin-Irgasan-Novobiocin Agar (CIN)					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (CCTACGGGAGGCAGCAG) and 926R (CCGTCAATTCCTTTRAGTTT)	(Ito *et al* 2019)
Chemically Defined Medium (CDM) (2x concentrate)					Anaerobic atmosphere 37°C 2 days	16S rRNA gene sequencing Regions: V3-V4	(Peterson *et al* 2022)
					Anaerobic atmosphere 37°C 3-4 days	16S rRNA gene sequencing Regions: V3-V4	(Peterson *et al* 2018)

Chocolate Agar (CHOC)					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (AGAGTTTGATCCTGGCTCAG) and 927r (CCGTCAATTC CTTTRAGTTT)	(Lau *et al* 2016)
Chocolate Agar (Chocopasteurised)					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (CCTACGGGAGGCAGCAG) and 926R (CCGTCAATTCCTTTRAGTTT)	(Ito *et al* 2019)
					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (CCTACGGGAGGCAGCAG-) and 926R (CCGTCAATTCCTTTRAGTTT)	(Ito *et al* 2019)
Chocolate Agar + PolyViteX (CPVX)					Aerobic atmosphere with 5% CO_2 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
		✓			Anaerobic and aerobic atmosphere 37 °C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)
					Aerobic atmosphere with 5% CO_2 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Aerobic atmosphere with 5% CO_2 37°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (GAATTGACGGGGRCCC) and 1391R (GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)
Christensenella Medium (CHRIS)		✓			Anaerobic and aerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)

Chromocult Agar					Not available	16S rRNA gene sequencing Regions: V3-V4	(Carroccio *et al* 2021)
Clostrisel Agar (CLOS)					Anaerobic atmosphere 37°C 72 hours	Bacteria morphology, Gram staining, catalase and oxidase tests, and growth under selective conditions	(Drago *et al* 2012)
Columbia Agar	✓				Aerobic atmosphere 37°C 3 days	MALDI-TOF MS 16S rRNA gene sequence Regions: V4-V5	(Samb-Ba *et al* 2014)
	✓				Anaerobic atmosphere 37°C 3 days	MALDI-TOF MS 16S rRNA gene sequence Regions: V4-V5	(Samb-Ba *et al* 2014)
Columbia Agar +	✓				Anaerobic atmosphere 37°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (GAATTGACGGGGRCCC) and 1391R (GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)
	✓				Aerobic atmosphere 28°C 14 days Growth at different temperatures: 22, 30, 37 and 56°C	16S rRNA gene sequencing	(Dubourg *et al* 2016)
	✓				Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS 16S rRNA gene sequence	(Pfleiderer *et al* 2013)
					Anaerobic atmosphere 28°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS 16S rRNA gene sequence	(Pfleiderer *et al* 2013)
					Microaerophile atmosphere 37°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (GAATTGACGGGGRCCC) and 1391R (GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)
	✓				Anaerobic atmosphere 37°C 4 days	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (GAATTGACGGGGRCCC) and 1391R (GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)
					Anaerobic atmosphere 37°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (GAATTGACGGGGRCCC) and 1391R (GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)

					Aerobic atmosphere 28°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (GAATTGACGGGGRCCC) and 1391R (GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)
	✓				Anaerobic atmosphere 37°C 5 days	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (GAATTGACGGGGRCCC) and 1391R (GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)
					Aerobic atmosphere 28°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (GAATTGACGGGGRCCC) and 1391R (GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)
					Aerobic atmosphere 28°C 48 hours	MALDI-TOF MS 16S rRNA gene sequence	(Samb-Ba *et al* 2014)
	✓				Anaerobic atmosphere 37°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (GAATTGACGGGGRCCC) and 1391R (GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)
					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
					Anaerobic atmosphere 28°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
					Microaerophilic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)

	✓				Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
	✓				Aerobic atmosphere 45°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
	✓				Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
					Anaerobic atmosphere 37°C 48 hours	16S rRNA gene sequencing	(Yimagou *et al* 2020)
	✓		✓	✓	Aerobic and Anaerobic atmosphere 37°C 28°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
	✓				Anaerobic atmosphere 37°C 4 days	MALDI-TOF MS	(Traore *et al* 2019)
					Aerobic atmosphere with 2.5% CO_2 37°C 5 days	MALDI-TOF MS 16S rRNA gene sequence	(Samb-Ba *et al* 2014)
				✓	Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS 16S rRNA gene sequencing 18S rRNA amplification and clone sequencing	(Dubourg *et al* 2013)
				✓	Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS 16S rRNA gene sequencing 18S rRNA amplification and clone sequencing	(Dubourg *et al* 2013)

					Anaerobic atmosphere 37°C 2 days	16S rRNA gene sequence analysis Whole-genome sequencing	(Ngom *et al* 2020)
	✓			✓	Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS 16S rRNA gene sequencing 18S rRNA amplification and clone sequencing	(Dubourg *et al* 2013)
				✓	Aerobic atmosphere 37°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence	(Lagier *et al* 2012)
					Anaerobic atmosphere 37°C 5 days	16S rRNA gene sequencing	(Goldman *et al* 2022)
					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing	(Lau *et al* 2016)
		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing	(López-Moreno *et al* 2022)
		✓			Anaerobic atmosphere 37°C 24-72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
	✓	✓			Aerobic and anaerobic atmosphere 37°C 24-72 hours	MALDI-TOF MS 16S rRNA gene sequencing	(Afouda *et al* 2020)
	✓				Anaerobic atmosphere 37°C 72 hours	MALDI-TOF MS 16S rRNA gene sequencing	(Benabdelkader *et al* 2020)
	✓				Anaerobic atmosphere 37°C 24-72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
Columbia NaladixicAcid (CNA) Agar	✓				Anaerobic atmosphere 37°C 24-72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)

					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (AGAGTTTGATCCTGGCTCAG) and 927r (CCGTCAATTC CTTTRAGTTT)	(Lau *et al* 2016)
Columbia NaladixicAcid (CNA) Agar + Sheep Blood 5%					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5'CCTACGGGAGGCAGCAG-3') and 926R (5'CCGTCAATTCCTTTRAGTTT-3')	(Ito *et al* 2019)
Columbia Agar Liquid Medium + Sheep Blood 5% (COS)		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)
Cooked Meat Agar (BEEF)					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (AGAGTTTGATCCTGGCTCAG) and 927r (CCGTCAATTC CTTTRAGTTT)	(Lau *et al* 2016)
Cycloserine Cefoxitin Fructose Agar (CCFA)					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (CCTACGGGAGGCAGCAG) and 926R (CCGTCAATTCCTTTRAGTTT)	(Ito *et al* 2019)
De Man, Rogosa, and Sharpe Agar (MRS)					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (AGAGTTTGATCCTGGCTCAG) and 927r (CCGTCAATTC CTTTRAGTTT)	(Lau *et al* 2016)
					Anaerobic atmosphere 37°C 72 hours	Bacteria morphology, Gram staining, catalase and oxidase tests, and growth under selective conditions	(Drago *et al* 2012)
De Man Rogosa and Sharpe (MRS) Agar with added 0.4% (w/v) CaCO3					CO_2 incubator (5% CO_2) 37°C 48 hours	16S rRNA gene sequencing Primers/Sequences: SU forward (CAC CAA CAG AGT TTG ATC CTG GCT CAG) and HDA2 reverse (GTA TTA CCG CGG CTG CTG GCA)	(Sornplang *et al* 2016)

Defined Base Medium						16S rRNA gene sequencing Regions: V3-V4 Primers/Sequences: S-D-Bact-0341-b-S-17 Forward (TCGTCGGCAGCGTCAGATGTGTAT AGAGACAGCCTACGGGNGGCWGCAG) and S-D-Bact-0785-a-A-21 Reverse (GTCTCGTGGGCTCGGAGATGTGTAT AGAGACAGGACTACHVGGGTATCTAATCC)	(Adamberg *et al* 2020)
Deoxycholate Agar (DOC)					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (AGAGTTTGATCCTGGCTCAG) and 927r (CCGTCAATTC CTTTRAGTTT)	(Lau *et al* 2016)
Drigalski Lactose Agar (BTB)					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (CCTACGGGAGGCAGCAG-) and 926R (CCGTCAATTCCTTTRAGTTT)	(Ito *et al* 2019)
DSMZ Culture Medium					Not available	16S rRNA gene sequencing SDArch0333aS1559TCCAGGCCCTACGG G39 and SDArch0958aA1959YCCGGCGTTGAMTC CAATT39	(Khelaifia *et al* 2013)
Eosin-Methylene Blue Agar (EMB)					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Fastidious Anaerobe Agar (FAA)					Anaerobic atmosphere 37°C 5 days	16S rRNA gene sequencing RegionS: V4- V5	(Goldman *et al* 2022)
					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (5'-AGAGTTTGATCCTGGCTCAG-3') and 927r (5'-CCGTCAATTC CTTTRAGTTT-3')	(Lau *et al* 2016)

					Anaerobic atmosphere 37°C 3-7 days	16S rRNA gene sequencing primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) were used to amplify part of the 16S rRNA gene	(Fenn *et al* 2017)
Fastidious Anaerobe Agar + 5% (v/v) Defibrinated Sheep Blood (FAA blood)					Anaerobic atmosphere 37°C 3-7 days	16S rRNA gene sequencing primers 27F (AGAGTTTGATCMTGGCTCAG-3') and 1492R (TACGGYTACCTTGTTACGACTT-3') were used to amplify part of the 16S rRNA gene	(Fenn *et al* 2017)
Gifu Anaerobe Media (GAM)					Anaerobic atmosphere 37°C 7 days	16S rRNA gene sequencing Region: V6 Primers/Sequences: 8F (AGAGTTTGATCC TGGCTCAG) and 1391R (GACGGGCGGTGTGTRCA)	(Rettedal *et al* 2014)
			✓		Anaerobic atmosphere 37°C 5 days	16S rRNA gene sequencing	(Li *et al* 2019)
		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)
					Anaerobic atmosphere 37°C 7 days	16S rRNA gene Region: V6 Primers/Sequences: 8F (AGAGTTTGATCC TGGCTCAG) and 1391R (GACGGGCGGTGTGTRCA)	(Rettedal *et al* 2014)
					Anaerobic atmosphere 37°C 48 hours	16S rRNA gene sequencing	(Chen *et al* 2019)
Gifu Anaerobic Media gellan (GAMg)		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing	(López-Moreno *et al* 2022)
Gifu Anaerobe Media (GAM), modified					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5'CCTACGGGAGGCAGCAG-3') and 926R (5'CCGTCAATTCCTTTRAGTTT-3')	(Ito *et al* 2019)
					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5'CCTACGGGAGGCAGCAG-3') and 926R (5'CCGTCAATTCCTTTRAGTTT-3')	(Ito *et al* 2019)

Gifu Anaerobe Media (GAM), diluted, modified					Anaerobic atmosphere 37°C 7 days	16S rRNA gene sequencing Region: V6 Primers/Sequences: 8F (AGAGTTTGATCC TGGCTCAG) and 1391R (GACGGGCGGTGTGTRCA)	(Rettedal *et al* 2014)
Glutamate Starch Phenol-Red aAgar (GSP) + Penicillin-G 60 g/L				✓	Not available	16S rRNA gene sequencing Regions: V3-V4	(Carroccio *et al* 2021)
Gram Negative Medium					Anaerobic atmosphere Incubation temperature and time: not available	16S rRNA gene sequencing	(Zou *et al* 2019)
Gut Microbiota Medium (GMM)					Anaerobic atmosphere 37°C 5 days	16S rRNA gene sequencing Regions: V4-V5	(Goldman *et al* 2022)
					Anaerobic atmosphere 37°C 48 hours	16S rRNA gene sequencing Region: V4	(Chen *et al* 2019)
					Anaerobic atmosphere 37°C 7 days	16S rRNA gene sequencing Region: V6 Primers/Sequences: 8F (AGAGTTTGATCC TGGCTCAG) and 1391R (GACGGGCGGTGTGTRCA)	(Rettedal *et al* 2014)
	✓		✓	✓	Anaerobic atmosphere 37°C 48-72 hours	MALDI-TOF MS 16S rRNA gene sequencing Whole genome sequencing	(Fleming *et al* 2021)
					Anaerobic atmosphere 37°C 7 days Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Region: V2 16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5′CCTACGGGAGGCAGCAG-3′) and 926R (5′CCGTCAATTCCTTTRAGTTT-3′)	(Ito *et al* 2019)
					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 927r (5′-CCGTCAATTC CTTTRAGTTT-3′)	(Lau *et al* 2016)
Haemophilus Test Medium (HTM)					Aerobic atmosphere with 2.5% CO_2 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)

					Aerobic atmosphere with 2.5% CO_2 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Hektoen Enteric Agar (HEA)					Aerobic atmosphere 35°C Overnight	MALDI-TOF MS Mass spectrometry	(He *et al* 2010)
Kanamycin Vancomycin Laked Blood Agar (KVLB)					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 927r (5′-CCGTCAATTC CTTTRAGTTT-3′)	(Lau *et al* 2016)
Lactobacilli MRS Agar (MRS) Mitis					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers/Sequences: 341F (5′CCTACGGGAGGCAGCAG-3′) and 926R (5′CCGTCAATTCCTTTRAGTTT-3′)	(Ito *et al* 2019)
Lactose Agar with Bromcresol Purple (BCP)					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
LAMVAB Medium					Aerobic atmosphere 37°C 48 hours	MALDI-TOF MS 16S rRNA gene sequence Regions: V4-V5	(Samb-Ba *et al* 2014)
					Anaerobic atmosphere 37°C 48 hours	MALDI-TOF MS 16S rRNA gene sequence Regions: V4-V5	(Samb-Ba *et al* 2014)
Listeria Enrichment Broth (Listeria)					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5′CCTACGGGAGGCAGCAG-3′) and 926R (5′CCGTCAATTCCTTTRAGTTT-3′)	(Ito *et al* 2019)
Luria-Bertani (LB) Medium					Anaerobic atmosphere Incubation temperature and time: not available	16S RNA gene sequencing	(Zou *et al* 2019)

M17 Agar					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
					Not available	16S rRNA gene sequencing Regions: V3-V4	(Carroccio *et al* 2021)
					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
M9 Minimal Media					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (5'-AGAGTTTGATCCTGGCTCAG-3') and 927r (5'-CCGTCAATTC CTTTRAGTTT-3')	(Lau *et al* 2016)
					Anaerobic atmosphere 37°C 7 days	16S rRNA gene sequencing Region: V6 Primers/Sequences: 8F (5'-AGAGTTTGATCC TGGCTCAG-3') and 1391R (5'-GACGGGCGGTGTGTRCA-3')	(Rettedal *et al* 2014)
MacConkey Agar (MAC)					Aerobic atmosphere 35°C Overnight Room temperature 48 hours	MALDI-TOF MS	(He *et al* 2010)
					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (5'-AGAGTTTGATCCTGGCTCAG-3') and 927r (5'-CCGTCAATTC CTTTRAGTTT-3')	(Lau *et al* 2016)
					Not available	16S rRNA gene sequencing Regions: V3-V4	(Carroccio *et al* 2021)
	✓				Aerobic atmosphere 37°C 1 day	MALDI-TOF MS 16S rRNA gene sequence RegionS: V4-V5 region	(Samb-Ba *et al* 2014)
McConkey Agar No. 3 and Slanetz and Bartley Agar			✓		Aerobic atmosphere 37°C 1, 2 or 3 days	Statistical analyses	(Mikkelsen *et al* 2015)

MacConkey Agar (MAC) + Sorbitol (MACS)					Aerobic atmosphere 35°C Overnight	MALDI-TOF MS	(He *et al* 2010)
MacConkey Agar (MAC) for Enterobacteriaceae					Aerobic atmosphere 37°C 24 hours	Bacteria morphology, Gram staining, catalase and oxidase tests, and growth under selective conditions	(Drago *et al* 2012)
Man Rogosa Sharp Agar (MRS)					Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)
					Aerobic and anaerobic atmosphere 37°C 72 hours	Whole Genome Sequencing (WGS) Phylogenomic identification Specific gene-encoding searches	(López-Moreno *et al* 2022)
					Aerobic and anaerobic atmosphere 37°C 72 hours	16S RNA gene sequencing Complete 16S RNA gene sequencing of selected bacterial strains by Sanger method	(Torres-Sanchez *et al* 2021)
					Not available	16S rRNA gene sequencing Regions: V3-V4	(Carroccio *et al* 2021)
	✓				Anaerobic atmosphere 37°C 24 to 72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
		✓			Anaerobic atmosphere 37°C 24 to 72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
					Anaerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
Mannitol Salt Agar (MSA)					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 927r (5′-CCGTCAATTC CTTTRAGTTT-3′)	(Lau *et al* 2016)

Marine Agar (MA)					Aerobic atmosphere 28°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Aerobic atmosphere 37°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (5'-GAATTGACGGGGRCCC) and 1391R (5'-GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)
					Aerobic atmosphere 28°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Marine Broth (MB)		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)
McKay Agar (MK)					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (5’-AGAGTTTGATCCTGGCTCAG-3’) and 927r (5’-CCGTCAATTC CTTTRAGTTT-3’)	(Lau *et al* 2016)
MOD 2 Agar					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
MPYG Medium					Anaerobic atmosphere Incubation temperature and time: not available	16S rRNA gene sequencing	(Zou *et al* 2019)

Mueller Hinton Agar (MH)					Anaerobic atmosphere Incubation temperature and time: not available	16S rRNA gene sequencing	(Zou *et al* 2019)
					Aerobic atmosphere 37°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (5'-GAATTGACGGGGRCCC) and 1391R (5'-GACGGGCGGTGWGTRCA)	(Lagier *et a*l 2012)
					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5'CCTACGGGAGGCAGCAG-3') and 926R (5'CCGTCAATTCCTTTRAGTTT-3')	(Ito *et al* 2019)
					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Mueller Hinton Agar (MH) + Vancomycin 50 µg/L			✓		Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
			✓		Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Orange Serum Agar					Aerobic atmosphere 37°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (5'-GAATTGACGGGGRCCC) and 1391R (5'-GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)
					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)

					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Phenylethyl Alcohol Agar (PEA)					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing	(Lau *et al* 2016)
Phenylethyl Alcohol Agar + Sheep Blood 5% (PEA)					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5'CCTACGGGAGGCAGCAG-3') and 926R (5'CCGTCAATTCCTTTRAGTTT-3')	(Ito *et al* 2019)
Plate Count Agar					Not available	16S rRNA gene sequencing Regions: V3-V4	(Carroccio *et al* 2021)
			✓		Aerobic atmosphere 37°C 1, 2 or 3 days	Statistical analyses of colony numbers	(Mikkelsen *et al* 2015)
Potato Dextrose Agar					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5'CCTACGGGAGGCAGCAG-3') and 926R (5'CCGTCAATTCCTTTRAGTTT-3')	(Ito *et al* 2019)
Reasoner's 2A Agar (R2A)					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Reinforced Clostridial Agar (RCM)		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)

	✓				Anaerobic atmosphere 37°C 24 to 72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
		✓			Anaerobic atmosphere 37°C 24 to 72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
R-Medium (RM)		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)
	✓				Anaerobic atmosphere 37°C 24 to 72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
Rogosa Agar + 1.32 mL/L Glacial Acetic Acid					Not available	16S rRNA gene sequencing Regions: V3-V4	(Carroccio *et al* 2021)
Sabouraud Agar (SAB)					Not available	16S rRNA gene sequencing Region: 16S RDNA gene Primers/Sequences: SDArch0333aS15-59TCCAGGCCCTACGGG-39 and SDArch0958aA1959YCCGGCGTTGAM TCCAATT-39	(Khelaifia *et al* 2013)
					Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Sabouraud Agar (SAB) + Cloramp Henicol 500 mg/L					Aerobic atmosphere 37°C 48 hours	Bacteria morphology, Gram staining, catalase and oxidase tests, and growth under selective conditions	(Drago *et al* 2012)
Salivarius Agar (Mitis) DHL Agar (DHL)					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5'CCTACGGGAGGCAGCAG-3') and 926R (5'CCGTCAATTCCTTTRAGTTT-3')	(Ito *et al* 2019)

Schaedler Agar	✓		✓		Anaerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Anaerobic atmosphere 37°C 24 hours, 48 hours then once a week for up to 2 months	MALDI-TOF MS 16S rRNA gene sequence Region: V6 Primers: 917F (5'-GAATTGACGGGGRCCC) and 1391R (5'-GACGGGCGGTGWGTRCA)	(Lagier *et al* 2012)
	✓				Anaerobic atmosphere 37°C 24 to 72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)
					Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Schaedler Agar + Kanamycin/ Vancomycin			✓		Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
			✓		Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
			✓		Anaerobic atmosphere 37°C 72 hours	Bacteria morphology, Gram staining, catalase and oxidase tests, and growth under selective conditions	(Drago *et al* 2012)
Schaedler Agar + 5% Blood					Anaerobic atmosphere 37°C 72 hours	Bacteria morphology, Gram staining, catalase and oxidase tests, and growth under selective conditions	(Drago *et al* 2012)

Selenite Broth (Selenite)					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5'CCTACGGGAGGCAGCAG-3') and 926R (5'CCGTCAATTCCTTTRAGTTT-3')	(Ito *et al* 2019)
Slanetz and Bartley Agar (SB)					Not available	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5'CCTACGGGAGGCAGCAG-3') and 926R (5'CCGTCAATTCCTTTRAGTTT-3')	(Carroccio *et al* 2021)
					Aerobic atmosphere 37°C 24 hours	Bacteria morphology, Gram staining, catalase and oxidase tests, and growth under selective conditions	(Drago *et al* 2012)
Thayer-Martin Selective Agar (TM)					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5'CCTACGGGAGGCAGCAG-3') and 926R (5'CCGTCAATTCCTTTRAGTTT-3')	(Ito *et al* 2019)
Thioglycolate + Agar + 5% Human Blood					Microaerophilic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
					Microaerophilic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
Tomato Juice Agar (Tomato)					Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing Regions: V3-V4 Primers: 341F (5'CCTACGGGAGGCAGCAG-3') and 926R (5'CCGTCAATTCCTTTRAGTTT-3')	(Ito *et al* 2019)
Tryptic Soy Agar (TSA)					Anaerobic atmosphere 37°C 5 days 5% CO_2 atmosphere 37°C 3 days	16S rRNA gene sequencing Region: V3 Primers/Sequences: 8f (5'-AGAGTTTGATCCTGGCTCAG-3') and 927r (5'-CCGTCAATTC CTTTRAGTTT-3')	(Lau *et al* 2016)

					Anaerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
					Aerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
Tryptic Soy Agar (TSA) + 5% Sheep Blood					10% CO_2-enriched air 37°C 24 hours	Bacteria morphology, Gram staining, catalase and oxidase tests, and growth under selective conditions	(Drago *et al* 2012)
	✓		✓	✓	Aerobic atmosphere 28°C 24-72 hours Anaerobic atmosphere 37°C 24-72 hours	MALDI-TOF MS Whole genome sequencing	(Fleming *et al* 2021)
Trypticase Soy Broth (TSB)		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)
Wilkins Chalgren Agar (WC)					Aerobic atmosphere 37 °C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)

	✓				Anaerobic atmosphere 37°C 24-72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
		✓			Anaerobic atmosphere 37°C 24-72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
					Aerobic atmosphere 37°C 2 days, 1 week, 2 weeks and 1 month	MALDI-TOF MS Clone sequencing 16S rRNA gene sequencing Region: V6 Primers/Sequences: FD1 (AGA GTT TGA TCC TGG CTC AG) and RP2 (ACG GCT ACC TTG TTA CGA CTT)	(Dubourg *et al* 2013)
			✓		Anaerobic atmosphere 37°C 2 or 3 days	Statistical analyses	(Mikkelsen *et al* 2015)
Wilkins Chalgren Agar (WC) + GN Selective Supplements and Defibrinated Sheep Blood					Not available	16S rRNA gene sequencing	(Carroccio *et al* 2021)
Wilkins Chalgren Agar (WC) + Soya Peptone Lcysteine, Tween® 80 Mupirocin and Glacial Acetic Acid					Anaerobic atmosphere 37°C 3 days	16S RNA gene sequencing	(Goodman *et al* 2011)
Wilkins Chalgren Agar (WC) + Sheep Blood			✓		Anaerobic atmosphere 37°C Incubation time: not available	MALDI-TOF MS	(Caputo *et al* 2015)
XD Medium					Anaerobic atmosphere Incubation temperature and time: not available	16S RNA gene sequencing	(Zou *et al* 2019)
YCFAG Agar					Anaerobic atmosphere 37°C 3-7 days	16S rRNA gene sequencing primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') were used to amplify part of the 16S rRNA gene	(Fenn *et al* 2017)
Yeast Casitone Fatty Acids Agar (YCFA)					Anaerobic atmosphere 37°C 5 days	16S rRNA gene sequencing Regions: V4-V5	(Goldman *et al* 2022)

	✓				Aerobic atmosphere 37°C 24 hours Anaerobic atmosphere 37°C 48 hours	MALDI-TOF MS 16S rRNA gene sequencing Primers: 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′)	(Hou *et al* 2019)
	✓				Anaerobic atmosphere 37°C 24-72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
		✓			Anaerobic atmosphere 37°C 24-72 hours	MALDI-TOF MS Metagenomics	(Diakite *et al* 2019)
	✓				Aerobic atmosphere 37°C 1 day Anaerobic atmosphere 37 °C 3 days	MALDI-TOF MS 16S rRNA gene sequencing Region: V4 Primers/Sequences: (27F: 5-AGAGTTTGATCCTGGCTCAG-3; 1492R: 5-GGTTACCTTGTTACGACTT-3)	(Chang *et al* 2019)
		✓			Anaerobic atmosphere 37°C 24 hours	16S rRNA gene sequencing	(Forster *et al* 2019)
		✓			Aerobic and anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Primers/Sequences: 16F27(AGAGTTTGATCMTGGCTCAG), 1525R (AAGGAGGTGATCCAGCC), F357(CTCCTACGGGAGGCAGCA), R519 (GWATTACCGCGGCKGCTG) and F915 (GGGCCCGCACAAGCGGTGG)	(López-Moreno *et al* 2022)
Yeast Casitone Fatty Acids Agar (YCFA) + 0.002 g/mL−1 each of Glucose, Maltose and Cellobios		✓			Anaerobic atmosphere 37°C 72 hours	16S rRNA gene sequencing Regions: V1-V2 Primers/Sequences: 27F (AATGATACGGCGACCACCGAGATCTACAC) and 338R CAAGCAGAAGACGGCATACGAGAT	(Browne *et al* 2016)
Yeast Casitone Fatty Acids Agar (YCFA) + Starch					Anaerobic atmosphere 37°C 5 days	16S rRNA gene sequencing Regions: V4-V5	(Goldman *et al* 2022)

[1]The preincubation methods can include preincubation in either blood cultures, marine broth, BHI or Trypticase Soy Broth.

Note: Identification methods have been provided where this information was available.

[2]From the study by Lagier *et al* (2012) only the 20 best culture conditions that facilitated the identification of 73% of the bacterial species were listed in this table.

Less commonly used media

Some researchers have focussed their efforts on isolating novel bacterial species using specific enriched media. An example of this approach comes from the work undertaken using the Yeast Casitone Fatty Acids Agar (YCFA) based culturing approach of Browne *et al* in 2016 (Browne *et al* 2016). This medium is enriched and has the nutrients yeast extract and pancreatic digest of casein added to encourage the growth of gut anaerobes. This medium has been effective in demonstrating that a high proportion of bacteria which are notoriously difficult to grow within the faecal population such as anaerobes and spore-forming bacteria can still be cultured in the laboratory under certain conditions.

Furthermore, bacteria cultured in another study (Browne *et al* 2016) included 90 species from the Human Microbiome Project's list of previously uncultured bacteria, supporting the concept that most gut microbiota can be cultured given the appropriate conditions (Browne *et al* 2016).

Other examples of less commonly used media include studies by Goodman *et al* (2011) who cultivated 50% of a donor's microbiota, a total of 316 species of the identified 632 distinct 97% ID operational taxonomic units (OTU), by plating diluted faecal material on plates containing pre reduced, nonselective Gut Microbiota Medium (GMM) with a 7-day incubation period at 37°C under an atmosphere of 75% nitrogen, 20% carbon dioxide, and 5% hydrogen. The significant finding of this study was the ability to culture a large number of gut microbiota using a single set of anaerobic culturing conditions and readily available reagents (Goodman *et al* 2011). Further to this in the first published culturomics study, different cultivation conditions resulted in the identification by high-throughput molecular techniques, of 99 bacterial species, 42 of which had never been previously been identified in the human gut, (Lagier *et al* 2012). Others such as Ito *et al* (2019), attempted to culture human gut bacteria by using 26 commercially available selective and non-selective media and 27 culture conditions in total. Some studies also showed that some bacteria which included representatives of the genera *Collinsella*, *Coprobacter*, *Butyricicoccus*, *Ihubacter*, *Desulfotomaculum*, *Anaeromassilibacillus*, *Anaerotruncus*, *Flintibacter* and *Faecalicoccus*, may require other growing conditions in order to be isolated (Ito *et al* 2019). These data clearly highlight the many challenges of relying solely on culture techniques to isolate gut microbiota even when using less commonly used media which may encourage the growth of gut microorganisms.

Supressing the growth of fast-growing microorganisms

One challenge faced by microbiologists is the suppression of fast-growing microbiota when attempting to isolate slow growing species. For many years selective media and various culturing conditions have been used (Hou *et al* 2019). Approaches have also included selectivity with antibiotics and enrichment in blood culture bottles (Lagier *et al* 2012; Benabdelkader *et al* 2020). When using antibiotics for selectivity, it must be considered that the inhibition of one specific group of microorganisms could also cause the loss of other species as some may depend on factors produced by other microbes to grow (Cross *et al* 2019). In the studies of Duquenoy *et al* (2020) and Caputo *et al* (2015) the glycopeptide antibiotic vancomycin was used to inhibit the growth of predominant bacterial populations and as a result the gram-negative *Akkermansia muciniphila* was able to be isolated (Duquenoy *et al* 2020); Caputo *et al* 2015).

Less well known approaches include culturing conditions which include the addition of sterile rumen fluid or fresh stool extracts, in order to mimic the gut natural environment and thus encouraging the growth of gut bacteria (Dubourg *et al* 2013). By using these additions Lagier *et al* (2012 & 2016) were able to isolate 17 strains that were not previously isolated with other culturing conditions (Lagier *et al* 2012; Lagier *et al* 2016).

Other approaches include preincubation in an anaerobic blood culture bottle with thioglycolate with anaerobic incubation directly on Columbia Agar + 5% sheep blood and passive filtration using a membrane in Leptospira broth (Pfleiderer *et al* 2013). Another approach was filtration with successive membranes which is a technique that has been used for many years to selectively culture organisms by inhibiting the more common bacterial populations. In addition, the use of phages in selecting for difficult to isolate bacterial species has also been described e.g. *E. coli* lytic bacteriophages were grown with *E. coli* a common gut commensal, which resulted in the isolation of an unknown enterobacterial species (*Enterobacter massiliensis*) (Lagier *et al* 2012). New bacterial species such as *Herbaspirillum massiliense* (Lagier *et al* 2012a) and *Cellulomonas massiliensis* (Lagier *et al* 2012b) have been isolated using filtration. The addition of antibiotics is one of the most commonly used methods to select for specific bacteria and when added to culture media can be used to significantly modify the composition of the bacterial population and selectively isolate target species (Versluis *et al* 2019; Vlkova *et al* 2015).

Another approach reported in various studies, involved the use of ethanol treatment of faecal samples. Browne *et al* (2016) employed this selective approach to identify sporulated bacteria and to facilitate the isolation of the *Clostridium difficile* spores from a mixed bacterial population. Not surprisingly, the pre-treatment of faecal samples with ethanol (70% for 4 hrs at room temperature) was found to lead to the isolation of ethanol-resistant spore forming bacteria with vegetative bacterial cells in the same sample being destroyed (Browne *et al* 2016).

In another study, 18 standard culturing conditions with and without ethanol disinfection were evaluated (Afouda *et al* 2020). The ethanol disinfection led to the cultivation of 60 species that were not isolated under standard cultivation conditions. Remarkably, of all bacterial species isolated in the 11 samples, 68 were unique to the ethanol disinfection technique. In their study, they observed an enrichment in Ruminococcaceae, Bacteroidaceae and Lachnospiraceae species using this technique, however other genera such as *Bacillus*, *Clostridium*, *Blautia*, *Lactobacillus* and *Prevotella* seemed to be less affected (Afouda *et al* 2020).

The above approaches demonstrate that each technique has certain advantages, although more research is required to ascertain how these techniques compare with each other in isolating bacteria that are difficult to culture.

Encapsulation and incubation in microdroplets

Techniques such as encapsulation and incubation in microdroplets inside multi-well micro culture chips have been used recently to improve the cultivation of fastidious bacteria (Cross *et al* 2019). This technique mimics in vivo natural selection processes that can be encouraged when cells are compartmentalised, thereby encapsulating individual micro-organisms and protecting them from competition from other species. In order to create compartmentalisation, a biocompatible microfluidic double water-in-oil-water emulsion is used to generate droplets in which single organisms can grow (Terekhov *et al* 2017). The microdroplet environment protects the cells providing a stable environment for growth.

Microfluidic chips are used to generate these droplets containing cells, and the droplets can be visualised using a haemocytometer. This technology can also be used for co-culturing of species, where one organism will produce factors that encourage the growth of the other. One disadvantage of this approach however is that the isolation of new species is influenced by chance as it may not be possible to predict what factors will enhance the growth of a novel microbe.

This approach has been successfully used by researchers to isolate new bacterial species (Ma *et al* 2014b; Tan *et al* 2020). Ma *et al* (2014a) applied the approach to successfully isolate and culture *Bacteroides vulgatus*, a gut anaerobe, from a clinical sample. Their method involved the use of a microfluidic SlipChip device that allows single microbial cells to be stochastically confined and subsequently cultured within the microcompartments.

Another study found an increase of up to four times the bacterial representation in stool samples, including rare or slow-growing species, using the microfluidic platform compared to conventional cultivation plates (Watterson *et al* 2020). The cultivation of 2.8 times more bacterial taxa compared to traditional culture methods was reported in another paper and this allowed the researchers to test the abundance of carbohydrate-degrading gut bacteria which would have not been possible with traditional bacterial culture techniques (Villa *et al* 2020). Faecal bacteria were cultured on ethanol-sterilised PAO chips and the bacterial growth analysed by 16S rRNA gene sequencing by Versluis *et al* in 2019, and showed a large range of taxa (Versluis *et al* 2019).

Co-culturing techniques

While co-culturing has been reported previously (Goodman *et al* 2011) more recent approaches have involved growing pairs of bacteria based upon the principle that one "helper" bacterium will secrete factors that encourage the growth of the other fastidious bacterium, with the bacteria growing around the "helper" bacterial colonies due to the presence of these biochemical factors. This approach mostly involves the use of solid media and was demonstrated recently by Fenn *et al* (2017) who were able to identify eight taxonomically diverse pairs of bacteri, using this approach. Amongst the multiple helper strains, *Escherichia coli* was found to have the ability to promote the growth of all induced isolates (Fenn *et al* 2017). Interestingly in this study it was concluded that the menaquinone biosynthesis pathway was required to induce the growth of seven out of the eight isolated strains, suggesting that the presence of quinones in growth media could play an important role in the isolation of fastidious bacteria such as *Faecalibacterium* species.

Similarly, Tanaka and Benno (2015) used a co-culture technique using soft agar separated by a membrane filter. Interbacterial communication was promoted by the diffusion of shared soluble substances between the two bacterial colonies through the membrane filter. By using this technique, they were able to isolate several gut bacteria such as *Parabacteroides* spp..

A more recent study employed a co-culture-based technique to isolate and cultivate a new strain of *Methanobrevibacter smithii* from a liquid culture medium inoculated with faeces in a co-culture with *Bacteroides thetaiotaomicron*. This is an effective method for growing certain bacteria with one organism producing a gas (for example in this case hydrogen produced by *B. thetaiotaomicron*) that promotes the growth of the other organism (Traore *et al* 2019). The technique mimics conditions in the gut microbiome where several bacteria produce metabolites that affect other bacteria and could be further developed to support the isolation of other bacteria (Tanaka and Benno 2015). More research into this approach is required however to determine how various gut microbes interact with each other and how some may support the growth of other organisms.

Conclusions

Many studies reviewed in this paper focussed on the isolation of specific bacteria. In situations where identification of individual species or strains of bacteria is not required and the aim is to identify bacterial genera, isolation of consortia of bacteria could be appropriate.

Culturing gut bacteria is challenging, and if used as the primary tool for diagnosing GI infections may lead to clinically significant bacteria being missed in faecal samples. An important consideration for clinical microbiologists is that methods (e.g. use of antibiotics, special media) designed to prevent growth of non-target organisms could potentially lead to important pathogens being missed.

In recent years techniques such as PCR have been increasingly used for the diagnosis of bacterial infections, however, in many situations such as determining antibiotic sensitivity, culture continues to be important in clinical microbiology. Based upon the papers reviewed in this study, it is clear that there is still an important role for culturing bacteria from biological specimens such as faeces, particularly when trying to study novel microbes.While culturing is not required for detecting microorganisms in clinical samples, being able to grow difficult to culture microorganisms is crucial to being able to isolate and identify novel species into the future. Further research on improving methods to culture microorganisms including bacteria, that are difficult to grow under standard laboratory conditions, is therefore warranted.

References

Adamberg K, Raba G, Adamberg S 2020. Use of Changestat for Growth Rate Studies of Gut Microbiota. *Front Bioeng Biotechnol* 8:24.

Afouda P, Hocquart M, Pham TP, Kuete E, Ngom, II, Dione N, Valles C, Bellali S, Lagier JC, Dubourg G, Raoult D 2020. Alcohol pretreatment of stools effect on culturomics. *Sci Rep* 10:5190.

Alkanani AK, Hara N, Gottlieb PA, Ir D, Robertson CE, Wagner BD, Frank DN, Zipris D 2015. Alterations in Intestinal Microbiota Correlate With Susceptibility to Type 1 Diabetes. *Diabetes* 64:3510-20.

Belkaid Y, Hand TW 2014. Role of the microbiota in immunity and inflammation. *Cell* 157:121-41.

Bibbo S, Dore MP, Pes GM, Delitala G, Delitala AP 2017. Is there a role for gut microbiota in type 1 diabetes pathogenesis? *Ann Med* 49:11-22.

Benabdelkader S, Naud S, Lo CI, Fadlane A, Traore SI, Aboudharam G, La Scola B 2020. Parabacteroides pacaensis sp. nov. and Parabacteroides provencensis sp. nov., two new species identified from human gut microbiota. *New Microbes New Infect* 34:100642.

Bonnet M, Lagier JC, Raoult D, Khelaifia S 2020. Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. *New Microbes and New Infect* 34:100622-100622.

Browne HP, Forster SC, Anonye BO, Kumar N, Neville BA, Stares MD, Goulding D, Lawley TD 2016. Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. *Nature (London)* 533:543-546.

Caputo A, Dubourg G, Croce O, Gupta S, Robert C, Papazian L, Rolain JM, Raoult D 2015. Whole-genome assembly of Akkermansia muciniphila sequenced directly from human stool. *Biol Direct* 10:5.

Carroccio A, Celano G, Cottone C, Di Sclafani G, Vannini L, D'Alcamo A, Vacca M, Calabrese FM, Mansueto P, Soresi M, Francavilla R, De Angelis M 2021. WHOLE-meal ancient wheat-based diet: Effect on metabolic parameters and microbiota. *Dig Liver Dis* 53:1412-1421.

Conlon MA, Bird AR 2014. The impact of diet and lifestyle on gut microbiota and human health. *Nutrients* 7:17-44.

Cha KH, Lee EH, Yoon HS, Lee JH, Kim JY, Kang K, Park JS, Jin JB, Ko G, Pan CH 2018. Effects of fermented milk treatment on microbial population and metabolomic outcomes in a three-stage semi-continuous culture system. *Food Chem* 263:216-224.

Chang Y, Hou F, Pan Z, Huang Z, Han N, Bin L, Deng H, Li Z, Ding L, Gao H, Zhi F, Yang R, Bi Y 2019. Optimization of Culturomics Strategy in Human Faecal Samples. *Front Microbiol* 10:2891.

Chen H, Nwe PK, Yang Y, Rosen CE, Bielecka AA, Kuchroo M, Cline GW, Kruse AC, Ring AM, Crawford JM, Palm NW 2019. A Forward Chemical Genetic Screen Reveals Gut Microbiota Metabolites That Modulate Host Physiology. *Cell* 177:1217-1231 e18.

Cross KL, Campbell JH, Balachandran M, Campbell AG, Cooper CJ, Griffen A, Heaton M, Joshi S, Klingeman D, Leys E, Yang Z, Parks JM, Podar M 2019. Targeted isolation and cultivation of uncultivated bacteria by reverse genomics. *Nat Biotechnol* 37:1314-1321.

Diakite A, Dubourg G, Dione N, Afouda P, Bellali S, Ngom, II, Valles C, Million M, Levasseur A, Cadoret F, Lagier JC, Raoult D 2019. Extensive culturomics of 8 healthy samples enhances metagenomics efficiency. *PLoS One* 14:e0223543.

Drago L, Toscano M, Rodighiero V, De Vecchi E, Mogna G 2012. Cultivable and Pyrosequenced Faecal Microflora in Centenarians and Young Subjects. Journal of clinical gastroenterology 46 Proceedings From The International Meeting On Probiotics, Prebiotics & New Foods Held In Rome On September 11–13, 2011:S81-S84.

Dubourg G, Lagier JC, Robert C, Armstrong N, Couderc C, Fournier PE, Raoult D 2016. Risungbinella massiliensis sp. nov., a new member of Thermoactinomycetaceae isolated from human gut. *Antonie Leeuwenhoek* 109:773-84.

Duquenoy A, Bellais S, Gasc C, Schwintner C, Dore J, Thomas V 2020. Assessment of Gram- and Viability-Staining Methods for Quantifying Bacterial Community Dynamics Using Flow Cytometry. *Front Microbiol* 11:1469.

Fenn K, Strandwitz P, Stewart EJ, Dimise E, Rubin S, Gurubacharya S, Clardy J, Lewis K 2017. Quinones are growth factors for the human gut microbiota. *Microbiome* 5:161.

Fleming E, Pabst V, Scholar Z, Xiong R, Voigt AY, Zhou W, Hoyt A, Hardy R, Peterson A, Beach R, Ondouah-Nzutchi Y, Dong J, Bateman L, Vernon SD, Oh J 2021. Cultivation of common bacterial species and strains from human skin, oral, and gut microbiota. *BMC Microbiol* 21:278.

Forster SC, Kumar N, Anonye BO, Almeida A, Viciani E, Stares MD, Dunn M, Mkandawire TT, Zhu A, Shao Y, Pike LJ, Louie T, Browne HP, Mitchell AL, Neville BA, Finn RD, Lawley TD 2019. A human gut bacterial genome and culture collection for improved metagenomic analyses. *Nat Biotechnol* 37:186-192.

Goldman SL, Sanders JG, Yan W, Denice A, Cornwall M, Ivey KN, Taylor EN, Gunderson AR, Sheehan MJ, Mjungu D, Lonsdorf EV, Pusey AE, Hahn BH, Moeller AH 2022. Culture-enriched community profiling improves resolution of the vertebrate gut microbiota. *Mol Ecol Resour* 22:122-136.

Goodman AL, Kallstrom G, Faith JJ, Reyes A, Moore A, Dantas G, Gordon JI 2011. Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *Proc Natl Acad Sci U S A* 108:6252-7.

He Y, Li H, Lu X, Stratton CW, Tang YW 2010. Mass spectrometry biotyper system identifies enteric bacterial pathogens directly from colonies grown on selective stool culture media. *J Clin Microbiol* 48:3888-92.

Hou F, Chang Y, Huang Z, Han N, Bin L, Deng H, Li Z, Pan Z, Ding L, Gao H, Yang R, Zhi F, Bi Y 2019. Application of LpxC enzyme inhibitor to inhibit some fast-growing bacteria in human gut bacterial culturomics. *BMC Microbiol* 19:308.

Huang Y, Sheth R.U, Zhao S. et al. High-throughput microbial culturomics using automation and machine learning. *Nat Biotechnol* 41, 1424–1433 (2023). https://doi.org/10.1038/s41587-023-01674-2.

Ito T, Sekizuka T, Kishi N, Yamashita A, Kuroda M 2019. Conventional culture methods with commercially available media unveil the presence of novel culturable bacteria. *Gut Microbes* 10:77-91.

Khelaifia S, Raoult D, Drancourt M 2013. A versatile medium for cultivating methanogenic archaea. *PLoS One* 8:e61563.

Lau JT, Whelan FJ, Herath I, Lee CH, Collins SM, Bercik P, Surette MG 2016. Capturing the diversity of the human gut microbiota through culture-enriched molecular profiling. *Genome Med* 8:72.

Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, Bittar F, Fournous G, Gimenez G, Maraninchi M, Trape JF, Koonin EV, La Scola B, Raoult D 2012. Microbial culturomics: paradigm shift in the human gut microbiome study. *Clin Microbiol Infect* 18:1185-93.

Lagier J-C, Dubourg G, Million M, Cadoret F, Bilen M, Fenollar F, Levasseur A, Rolain J-M, Fournier P-E, Raoult D 2018. Culturing the human microbiota and culturomics. *Nat Rev Microbiol* 16:540-550.

Lagier J-C, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D 2015. Current and past strategies for bacterial culture in clinical microbiology. *Clin Microbiol Rev* 28:208-236.

Lagier J-C, Gimenez G, Robert C, Raoult D, Fournier P-E 2012a. Non-contiguous finished genome sequence and description of Herbaspirillum massiliense sp. nov. *Stand Genom Sci* 7:200-14.

Lagier JC, Khelaifia S, Alou MT, Ndongo S, Dione N, Hugon P, Caputo A, Cadoret F, Traore SI, Seck EH, Dubourg G, Durand G, Mourembou G, Guilhot E, Togo A, Bellali S, Bachar D, Cassir N, Bittar F, Delerce J, Mailhe M, Ricaboni D, Bilen M, Dangui Nieko NP, Dia Badiane NM, Valles C, Mouelhi D, Diop K, Million M, Musso D, Abrahao J, Azhar EI, Bibi F, Yasir M, Diallo A, Sokhna C, Djossou F, Vitton V, Robert C, Rolain JM, La Scola B, Fournier PE, Levasseur A, Raoult D 2016. Culture of previously uncultured members of the human gut microbiota by culturomics. *Nat Microbiol* 1:16203.

Lagier J-C, Ramasamy D, Rivet R, Raoult D, Fournier P-E 2012b. Non contiguous-finished genome sequence and description of Cellulomonas massiliensis sp. nov. *Stand Genom Sci* 7:258-270.

Lagkouvardos I, Overmann J, Clavel T 2017. Cultured microbes represent a substantial fraction of the human and mouse gut microbiota. *Gut microbes* 8:493-503.

Li J, Rettedal EA, van der Helm E, Ellabaan M, Panagiotou G, Sommer MOA 2019. Antibiotic Treatment Drives the Diversification of the Human Gut Resistome. *Genomics Proteomics Bioinformatics* 17:39-51.

López-Moreno A, Ruiz-Moreno Á, Pardo-Cacho J, Cerk K, Torres-Sánchez A, Ortiz P, Úbeda M, Aguilera M 2022. Culturing and Molecular Approaches for Identifying Microbiota Taxa Impacting Children's Obesogenic Phenotypes Related to Xenobiotic Dietary Exposure. *Nutrients* 14:241.

López-Moreno A, Torres-Sánchez A, Acuña I, Suárez A, Aguilera M 2021. Representative Bacillus sp. AM1 from Gut Microbiota Harbor Versatile Molecular Pathways for Bisphenol A Biodegradation. *Int J Mol Sci* 22:4952.

Lynch SV, Pedersen O 2016. The Human Intestinal Microbiome in Health and Disease. *N Engl J Med* 375:2369-2379.

Ma L, Datta SS, Karymov MA, Pan Q, Begolo S, Ismagilov RF 2014a. Individually addressable arrays of replica microbial cultures enabled by splitting SlipChips. *Integr Biol (Camb)* 6:796-805.

Ma L, Kim J, Hatzenpichler R, Karymov MA, Hubert N, Hanan IM, Chang EB, Ismagilov RF 2014b. Gene-targeted microfluidic cultivation validated by isolation of a gut bacterium listed in Human Microbiome Project's Most Wanted taxa. *PNAS* 111:9768-9773.

Mikkelsen KH, Frost M, Bahl MI, Licht TR, Jensen US, Rosenberg J, Pedersen O, Hansen T, Rehfeld JF, Holst JJ, Vilsboll T, Knop FK 2015. Effect of Antibiotics on Gut Microbiota, Gut Hormones and Glucose Metabolism. *PLoS One* 10:e0142352.

Moher D, Liberati A, Tetzlaff J, Altman DG 2008. Preferred reporting items for systematic reviews and meta-analyses; The PRISMA Statement. *PLoS Med 6* (2008), p. e1000097.

Ngom, II, Hasni I, Lo CI, Traore SI, Fontanini A, Raoult D, Fenollar F 2020. Taxono-genomics and description of Gordonibacter massiliensis sp. nov., a new bacterium isolated from stool of healthy patient. *New Microbes New Infect* 33:100624.

Pereira AC, Cunha MV 2020. An effective culturomics approach to study the gut microbiota of mammals. *Res Microbiol* 171:290-300.

Peterson CT, Perez Santiago J, Iablokov SN, Chopra D, Rodionov DA, Peterson SN. 2022. Short-Chain Fatty Acids Modulate Healthy Gut Microbiota Composition and Functional Potential. *Curr Microbiol* 79:128-128.

Peterson CT, Sharma V, Uchitel S, Denniston K, Chopra D, Mills PJ, Peterson SN 2018. Prebiotic Potential of Herbal Medicines Used in Digestive Health and Disease. *J Altern Complement Med* 24:656-665.

Pfleiderer A, Lagier JC, Armougom F, Robert C, Vialettes B, Raoult D 2013. Culturomics identified 11 new bacterial species from a single anorexia nervosa stool sample. *Eur J Clin Microbiol Infect Dis* 32:1471-81.

Rettedal EA, Gumpert H, Sommer MO 2014. Cultivation-based multiplex phenotyping of human gut microbiota allows targeted recovery of previously uncultured bacteria. *Nat Commun* 5:4714.

David Rojo, Celia Méndez-García, Beata Anna Raczkowska, Rafael Bargiela, Andrés Moya, Manuel Ferrer, Coral Barbas, Exploring the human microbiome from multiple perspectives: factors altering its composition and function, *FEMS Microbiology Reviews*, Volume 41, Issue 4, July 2017, Pages 453–478, https://doi.org/10.1093/femsre/fuw046.

Samb-Ba B, Mazenot C, Gassama-Sow A, Dubourg G, Richet H, Hugon P, Lagier JC, Raoult D, Fenollar F 2014. MALDI-TOF identification of the human Gut microbiome in people with and without diarrhea in Senegal. *PLoS One* 9:e87419.

Sommer MOA. 2015. Advancing gut microbiome research using cultivation. *Curr opin microbiol* 27:127-132.

Sornplang P, Sakulsawasdiphan K, Piyadeatsoontorn S, Surasorn B 2016. Antimicrobial susceptibility of lactic acid bacteria isolated from human and food-producing animal faeces in Khon Kaen Province, Thailand. *Trop Anim Health Prod* 48:1739-1745.

Tan JY, Wang S, Dick GJ, Young VB, Sherman DH, Burns MA, Lin XN 2020. Co-cultivation of microbial sub-communities in microfluidic droplets facilitates high-resolution genomic dissection of microbial 'dark matter'. *Integr Biol (Camb)* 12:263-274.

Tanaka Y, Benno Y 2015. Application of a single-colony coculture technique to the isolation of hitherto unculturable gut bacteria. *Microbiol Immunol* 59:63-70.

Terekhov SS, Smirnov IV, Stepanova AV, Bobik TV, Mokrushina YA, Ponomarenko NA, Belogurov AA Jr, Rubtsova MP, Kartseva OV, Gomzikova MO, Moskovtsev AA, Bukatin AS, Dubina MV, Kostryukova ES, Babenko VV, Vakhitova MT, Manolov AI, Malakhova MV, Kornienko MA, Tyakht AV, Vanyushkina AA, Ilina EN, Masson P, Gabibov AG, Altman S. Microfluidic droplet platform for ultrahigh-throughput single-cell screening of biodiversity. Proc Natl Acad Sci U S A. 2017 Mar 7;114(10):2550-2555. doi: 10.1073/pnas.1621226114. Epub 2017 Feb 15. PMID: 28202731; PMCID: PMC5347554.

Traore SI, Khelaifia S, Armstrong N, Lagier JC, Raoult D 2019. Isolation and culture of Methanobrevibacter smithii by co-culture with hydrogen-producing bacteria on agar plates. *Clin Microbiol Infect* 25:1561 e1-1561 e5.

Torres-Sanchez A, Pardo-Cacho J, Lopez-Moreno A, Ruiz-Moreno A, Cerk K, Aguilera M 2021. Antimicrobial Effects of Potential Probiotics of Bacillus spp. Isolated from Human Microbiota: In Vitro and In Silico Methods. *Microorganisms* 9.

Valdes AM, Walter J, Segal E, Spector TD 2018. Role of the gut microbiota in nutrition and health. *BMJ* 361:k2179.

Versluis D, de JBGT, Zoetendal EG, Passel M, Smidt H 2019. High throughput cultivation-based screening on porous aluminum oxide chips allows targeted isolation of antibiotic resistant human gut bacteria. *PLoS One* 14:e0210970.

Villa MM, Bloom RJ, Silverman JD, Durand HK, Jiang S, Wu A, Dallow EP, Huang S, You L, David LA 2020. Interindividual Variation in Dietary Carbohydrate Metabolism by Gut Bacteria Revealed with Droplet Microfluidic Culture. *mSystems* 5.

Vlkova E, Salmonova H, Bunesova V, Geigerova M, Rada V, Musilova S 2015. A new medium containing mupirocin, acetic acid, and norfloxacin for the selective cultivation of bifidobacteria. *Anaerobe* 34:27-33.

Watterson WJ, Tanyeri M, Watson AR, Cham CM, Shan Y, Chang EB, Eren AM, Tay S 2020. Droplet-based high-throughput cultivation for accurate screening of antibiotic resistant gut microbes. *Elife* 9..

Yimagou EK, Dione N, Ngom, II, Tall ML, Baudoin JP, Raoult D, Khalil JYB 2020. Parabacteroides bouchesdurhonensis sp. nov., a new bacterium isolated from the stool of a healthy adult. *New Microbes New Infect* 34:100639.

Zou Y, Xue W, Luo G, Deng Z, Qin P, Guo R, Sun H, Xia Y, Liang S, Dai Y, Wan D, Jiang R, Su L, Feng Q, Jie Z, Guo T, Xia Z, Liu C, Yu J, Lin Y, Tang S, Huo G, Xu X, Hou Y, Liu X, Wang J, Yang H, Kristiansen K, Li J, Jia H, Xiao L 2019. 1,520 reference genomes from cultivated human gut bacteria enable functional microbiome analyses. *Nat Biotechnol* 37:179-185.

REVIEW ARTICLE

Heparin induced thrombocytopenia: diagnosis, treatment, challenges and future of testing

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Abstract

Diagnosis of heparin induced thrombocytopenia (HIT) involves a comprehensive approach combining clinical assessment and laboratory investigations. The 4T score system is commonly utilized to assess the probability of HIT although its subjective nature and lack of sensitivity and specificity present limitations. Laboratory tests such as platelet activation assays and immunoassays are being explored to complement clinical judgment and enhance diagnostic accuracy. These tests have the potential to provide objective information to support or refute the diagnosis of HIT thereby improving patient outcomes by reducing the risk of both overdiagnosis and underdiagnosis.

Treatment of HIT primarily involves the prompt discontinuation of heparin therapy and initiation of alternative anticoagulation strategies. Direct oral anticoagulants (DOACs) and low molecular weight heparin (LMWH) such as danaproid and fondaparinux as well as parenteral direct thrombin inhibitors (DTIs) are commonly used alternatives; however careful consideration must be given to individual patient factors. Close monitoring for thrombotic complications and adequate management of ongoing anticoagulation are crucial elements of HIT treatment.

Challenges in HIT management include the need for rapid and reliable diagnostic tools, as well as the potential for misdiagnosis due to the overlapping clinical features of HIT with other conditions. The availability and cost of alternative anticoagulants can also pose challenges in resource-limited settings. Clear communication and interdisciplinary collaboration among healthcare providers are essential to ensure optimal management and patient safety.

The future of HIT testing holds promise with ongoing research and development. Advancements in laboratory assays aim to improve diagnostic precision and aid in the differentiation of HIT from other causes of thrombocytopenia. These evolving tests have the potential to enhance sensitivity and specificity, thereby reducing the risk of unnecessary treatment modifications and associated complications., The identification of novel biomarkers and genetic markers may further contribute to earlier detection and personalized management of HIT.

Keywords: ***HIT diagnosis, platelet factor 4, heparin, laboratory diagnosis, treatment***

Introduction

Heparin (H) is a common parenterally administered anticoagulant given to patients to treat or prevent thrombosis (Carpenè *et al* 2022). Some patients receiving heparin medication unfortunately develop a significant adverse pharmacological response – HIT (Warkentin and Greinacher 2004). HIT is an autoimmune disorder; the immune system of susceptible patients produces antibodies in reaction to heparin, in complex with a platelet component called platelet factor 4 (PF4). These antibody complexes can bind to platelets and cause their clearance, leading to a decline in the patient's platelet count and an increase in the risk of blood clots (Warkentin and Greinacher 2004).

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In affected patients, HIT symptoms can be ambiguous and HIT can possibly be mistaken for other medical conditions and therefore identifying HIT can be difficult (Greinacher and Warkentin 2006). Thrombocytopenia (low platelet

count), skin lesions, fever, and thrombotic events (blood clots) are some of the common symptoms of HIT (Greinacher and Warkentin 2006). These symptoms can also be present in other illnesses therefore it is crucial for clinicians to be aware of the possibility of HIT and to investigate this possibility further (Greinacher and Warkentin 2006).

HIT identification can occur with the use of laboratory testing. There are a wide variety of assays that can test for the possibility of HIT (PF4/H) antibodies in blood; HIT immunological screening assays. The enzyme-linked immunosorbent (ELISA) test is one of the commonly performed tests which detects IgG antibodies against PF4/H complexes (Arepally 2017) and is supplied by a variety of manufacturers/suppliers (Favaloro 2018). A positive ELISA test result indicates that the patient has antibodies against PF4/H complexes and thus may have HIT. One problem with ELISA testing is that although very sensitive to the presence of PF4/H, the assays take several hours to perform, and are therefore not suited to urgent or 'STAT' testing. There are several other immunological HIT-screening assay types that detects IgM and IgA isotypoes available from a wide number of manufacturers/suppliers. Among these, there are several 'rapid' screening assays that can be performed in 10-35 minutes, thereby offering the possibility of fast screening.

A diagnosis of pathological HIT cannot be confirmed exclusively based on a positive immunological assay, as some individuals can have antibodies to PF4/H complexes without developing pathological HIT (Arepally 2017). Characterization of PF4/H antibodies as pathological condition requires confirmation using a functional HIT assay. The serotonin release assay (SRA), for example, is one such assay, and can be performed to confirm the diagnosis of pathological HIT (Arepally 2017).

Currently in our New South Wales (NSW) Health Pathology (NSWHP) network, rapid HIT screen testing is only available in selected metropolitan laboratories. Laboratories that have AcuStar (Werfen) instruments can assess for PF4/H antibodies using the rapid HIT IgG test kit by chemiluminescence, but these instruments are only at specific sites. One laboratory within the NSWHP network alternatively uses the ELISA method, and another laboratory uses the particle gel immunoassay (PaGIA) Heparin/PF4 Antibody test kit by BIORAD. The challenge with these assays is that they are performed in batches. The PaGIA (BIORAD) assay is capable of detecting IgG, IgM and IgA isotypes. Delays in HIT diagnosis can unfortunately have critical impact for clinical care and treatment strategies for an already complicated and unwell patient cohort.

Pathophysiology of HIT

The incidence of HIT is estimated to be 0.2-5% in patients exposed to unfractionated heparin (UFH), while the incidence is lower (0.2-1%) in patients exposed to low molecular weight heparin (LMWH) (Warkentin and Greinacher 2004).

Several risk factors have been identified for the development of HIT, including the duration and intensity of heparin exposure, the route of administration, and underlying medical conditions. Some of the commonly reported risk factors include:

1. Previous exposure to heparin: Patients who have received heparin in the past are at a higher risk of developing HIT, with the risk increasing with each exposure (Arepally 2017).
2. Type of heparin: UFH is more likely to cause HIT compared to LMWH due to differences in their molecular weight and structure (Arepally 2017).
3. Dose and duration of heparin therapy: Higher doses of heparin and prolonged exposure are associated with an increased risk of developing HIT (Arepally 2017).
4. Patient characteristics: Certain medical conditions such as cancer, autoimmune diseases, and infections and older age can increase the risk of HIT (Arepally 2017).
5. Genetic factors: Certain genetic polymorphisms have been associated with an increased risk of HIT (Arepally 2017).

HIT may present in a variety of ways, from moderate to severe signs and symptoms. In most cases, the symptoms appear 5 to 10 days after starting heparin medication, although in rare circumstances, they may appear sooner rather than that (Arepally 2017). Common clinical features of HIT include the following:

1. Thrombocytopenia: One distinct sign of HIT is a drop in the platelet count.
2. Venous and arterial thrombosis: HIT may cause blood to clot; clots can occur in veins and arteries and may cause myocardial infarction (MI), pulmonary embolism (PE), stroke, or deep vein thrombosis (DVT) (Arepally 2017).
3. Skin lesions: Heparin-induced skin lesions, including erythematous macules, nodules, and necrotic lesions may appear on the skin of HIT patients (Arepally 2017).

4. Systemic signs and symptoms: HIT can also result in systemic signs and symptoms such as fever, chills, and malaise (Warkentin *et al* 2000).

HIT represents immune-mediated adverse reaction to heparin therapy that is characterised by the formation of antibodies, primarily IgG, against complexes of heparin (H) and platelet factor 4 (PF4) (Warkentin 2003). In pathological HIT, these PF4/H antibodies may cause platelet activation and aggregation, leading to a hypercoagulable state that can result in thrombosis, ranging from deep vein thrombosis (DVT) to life-threatening arterial thrombosis, including stroke and myocardial infarction (MI) (Greinacher 2015).

This process of platelet activation and clot formation can occur even in the absence of any prior exposure to heparin, as pre-existing antibodies to PF4 have been detected in a small proportion of the general population (Warkentin *et al* 2008). In the presence of heparin however, the formation of antibodies to the PF4/H complex is greatly increased, leading to a higher risk of thrombosis (Magnani 1993). The risk of developing pathological HIT is also influenced by other factors as listed above (Lo *et al* 2006).

Management and treatment

Pathological HIT is a potentially fatal illness that necessitates immediate diagnosis and treatment. The goals of treatment are to both prevent further thrombosis and decrease the risk of bleeding. There are several guidelines available to effectively manage and treat HIT (Warkentin *et al* 2008). Joseph *et al* (2019) have written a consensus guidelines formulated by members of Thrombosis and Haemostasis Society of Australia and New Zealand (THANZ). The first step in the treatment of HIT is to cease using heparin in any form. To prevent further thrombosis, cessation of heparin must be followed by initiation of alternative anticoagulant therapy; these may include factor Xa inhibitors or direct thrombin inhibitors (DTIs) (Linkins *et al* 2012). The use of DOACs as an alternative to heparin-based therapies has several advantages in HIT management due to their predictable pharmacokinetics, fixed dosing regimens, and lack of routine monitoring requirements (Linkins *et al* 2012). The agents argatroban, bivalirudin, and fondaparinux are a few examples of anticoagulants that can be used in place of heparin (Warkentin *et al* 2008). Another important step is to regularly monitor platelet count and coagulation parameters, for example the activated partial thromboplastin time (aPTT) and/or anti-Xa activity to help evaluate treatment effectiveness and adjust anticoagulant medication dosage (Warkentin *et al* 2008). Another requirement is to manage thrombotic complications: patients with HIT-associated thrombosis require therapeutic anticoagulation, which in some circumstances includes catheter-directed thrombolysis or surgical intervention (Warkentin *et al* 2008). Clinicians should avoid treating the thrombocytopenia using platelet transfusions as these could instead 'fuel the fire' and exacerbate thrombotic issues; consequently platelet transfusions are largely contraindicated in HIT (Arepally and Ortel 2006).

Laboratory investigations for HIT

Screening assays

Laboratory testing is essential for the diagnosis of HIT. There are currently a variety of methodologies and test available to screen for HIT PF4/H antibodies:

1. **Enzyme-linked immunosorbent assay (ELISA):** This assay is considered as the gold standard for HIT 'screening' due to its sensitivity and specificity. It is also a commonly used test to detect IgG antibodies present to heparin-PF4-complexes in the patient's serum This method utilises a 96-well plate that has been coated with heparin-PF4-complexes, which then will capture any HIT antibodies present from the patient's serum. Once any unbound proteins are washed away in the washing step, a secondary antibody labelled with an enzyme is added to identify the presence of captured HIT antibodies. Finally, after an incubation period, a colorimetric reaction is used to measure the amount of enzyme activity and as a result, the amount of HIT antibodies in the patient's serum. The intensity of the colour reaction is directly proportional to the concentration of the antibodies present in the sample (Warkentin and Greinacher 2004).

2. **Chemiluminescence immunoassay (CLIA) – AcuStar (Werfen):** This is a quick and automated chemiluminescence test that uses magnetic particles coated with PF4/polyvinyl sulfonate (PVS) to detect IgG associated with anti-PF4/heparin antibodies. The test involves incubation of magnetic particles with the patient's serum or citrated plasma, followed by a wash step. An isoluminol-labeled antihuman IgG antibody tracer is then added, which binds to the captured anti-PF4/heparin antibodies on the particles. After another incubation and washing, reagents that emit light are added, and the emitted light is directly proportional to the concentration of anti-PF4/heparin antibodies in the sample (Warkentin 2019). Results are available in about 30 minutes after preparation of the sample.

3. **Particle Gel Immunoassay (PaGIA) – Biorad:** This assay is a type of immunoassay that is based on the principle of antigen-antibody binding. In this test, heparin-PF4 complexes are bound to particles,

usually latex particles or beads, and used to detect HIT IgG, IgM and IgA antibodies in a patient's serum. The assay is performed by mixing a sample of patient serum with the heparin-PF4 particles. If HIT antibodies are present in the patient's serum, they will attach to the heparin-PF4 particles and create a complex. The next step is the addition of a gel matrix that contains anti-human IgG antibodies which will capture any complex formed by the HIT antibodies and heparin-PF4 particles. As a result, a visible gel agglutination ring appears, which indicates the presence of HIT antibodies in the patient's serum (Sachan *et al* 2013). Although PaGIA has lower sensitivity and specificity than ELISA, it can still be helpful in identifying heparin-dependent antibodies in patients with suspected HIT.

4. **Lateral flow immunoassay (LFIA):** LFIA is a rapid point-of-care test that uses a disposable test cartridge to detect HIT antibodies in patient serum. This test is less sensitive and specific than ELISA, but it provides rapid results and is more convenient for screening purposes (Gardiner *et al* 2014). The STic® Expert HIT kit (Diagnostica Stago, Asnieres, France) employs a lateral flow immunoassay technique to identify IgG antibodies that bind to PF4/polyanion complexes. These complexes are located within an evaluation card and the test takes 10-20 minutes to complete (Kumar *et al* 2019).

5. **Latex immunoassay (LIA):** This assay is still under the category immunoassays; it differs from typical immunoassays like the ELISAs in terms of how it operates. In the LIA, the presence of PF4/H reactive antibodies in a patient's plasma leads to the inhibition of particle agglutination due to competition with a monoclonal antibody that mimics HIT. This unique characteristic has led to the term "functionalized immunoassay" being associated with the LIA. The implication is that the LIA may offer diagnostic specificity that falls between the highly specific functional assays (such as the SRA) and the less specific PF4-dependent ELISAs (Warkentin *et al* 2017).

A summary of these assays in shown in Table 1.

Various antibody-capturing platforms, such as ELISA-based methods, particle gel, and turbidimetry, are utilized by immunoassays to detect anti-PF4-heparin antibodies. These immunoassays exhibit high sensitivity (exceeding 99%) which makes them suitable as an initial screening test for heparin-induced HIT (Sahu *et al* 2020). However, their diagnostic specificity is relatively low, ranging from 30% to 70%, primarily due to the occurrence of asymptomatic seroconversions.

This limitation results in a reduced positive predictive value.

Typically, only 10% to 50% of patients with positive immunoassay results possess platelet-activating antibodies associated with HIT. Despite this drawback, immunoassays offer the advantage of being routinely performed by most laboratories and provide rapid response times (Sahu *et al* 2020).

Nagler *et al* (2016) noted significant variations among different types of immunoassays regarding antibody specificity, thresholds and confirmation steps. Out of all the tests, only five demonstrated a combination of high sensitivity (> 95%) and high specificity (> 90%). These are the polyspecific enzyme-linked immunosorbent assay (ELISA) with an intermediate threshold (Genetic Testing Institute, Asserachrom), particle gel immunoassay, lateral flow immunoassay, polyspecific chemiluminescent immunoassay (CLIA) with a high threshold, and immunoglobulin G (IgG)-specific CLIA with a low threshold (Nagler *et al* 2016). Results at the borderline level (sensitivity 99.6%; specificity 89.9%) were observed for IgG-specific Genetic Testing Institute-ELISA with a low threshold. Tests with high thresholds (ELISA; IgG-specific CLIA), a combination of IgG specificity and intermediate thresholds (ELISA, CLIA), a confirmation step using high-dose heparin (ELISA), and particle immunofiltration assay demonstrated inadequate diagnostic accuracy (Nagler *et al* 2016).

Functional assays

HIT platelet activation assays such as the SRA and heparin-induced platelet activation (HIPA) test, measure platelet activity using the patient's serum and various concentrations of heparin (Refaai *et al* 2019). These tests are more specific for clinically significant HIT antibodies than the immunological 'screening' assays. They are difficult to perform however and are time-consuming, requiring expertise in specialized laboratories. They are typically performed after a positive immunoassay result has been obtained, which further delays the confirmation or exclusion of the HIT diagnosis (Refaai *et al* 2019).

1. **Serotonin release assay (SRA)** is a functional assay used for the laboratory diagnosis of pathological HIT. This assay measures the release of serotonin from platelets in response to heparin and the presence of HIT antibodies in the patient's serum (Warkentin 2019). The test is the gold standard for HIT diagnosis because it directly measures platelet activation.

   In the SRA test, donor platelets are incubated with the patient serum in the presence of heparin. The platelets are then washed and exposed to aggregating agents, which induces the release of

Table 1. *Summary of heparin-induced thrombocytopenia immunological assays (from Joseph* et al *2019).*

Assay	Results	Principle	Advantages	Disadvantages
ELISA	Quantitative	ELISA, PF4/H surface bound.	Optical density (OD) results and cut-off value. High negative predictive value. A confirmatory step using high heparin concentration is possible.	Long turnaround time due to tests performed in batches. Typically, not possible for an on-demand testing for a single patient.
CLIA	Quantitative	Chemiluminescence assay. Detects IgG antibodies associated with HIT.	On demand testing. Fast turnaround time. Very high negative predictive value.	Can only be performed using a specific manufacturer. High-priced reagents. Occasional false negatives.
LIA	Quantitative	Agglutination based assay using latex particles.	Quick turnaround time. Primarily detects IgG isotype.	Instrumentation is necessary. Limited to IgG detection. High cost.
PaGIA	Qualitative	Agglutination test incorporating heparin-PF4 coated coloured polystyrene particles. Capable of detecting IgG, IgM and IgA.	Fast turnaround time. Able to detect multiple immunoglobulin isotypes (IgG, IgM and IgA).	Must use a centrifuge from a specific manufacturer instrument/kit. Not IgG-specific. Result interpretation by visual examination and subjectivity.
LFIA	Qualitative	Specific for IgG antibody using qualitative immunochromatography.	Fast turnaround time (fastest of all heparin-induced thrombocytopenia assays). Very sensitive assay to IgG antibody.	Result interpretation done by visual assessment. Reduced positive predictive value (<50%). Not recommended for weak antibodies. Occasional false negatives. Limited to IgG detection.

serotonin. The amount of serotonin released is measured and compared to a reference. A positive HIT SRA result is usually identified by serum-induced platelet serotonin release at therapeutic heparin low dose of 0.1 U/mL but inhibited at high dose of heparin 100 U/mL . SRA is a highly specific test and has a low false positive rate. It is technically demanding and time-consuming and requires fresh donor platelets, which can limit its availability. The test is also requires use of radioisotopes and is more expensive than other HIT assays (Warkentin 2019).

The platelet serotonin release assay (SRA) was developed in 1986 at McMaster University in Hamilton, ON, Canada. This test established optimal conditions to activate platelets obtained from normal donors using serum or plasma from patients with HIT (Warkentin 2016). The platelets were carefully processed by washing them in a buffer with low pH, no calcium, and containing apyrase to prevent activation by adenosine diphosphate. They were then resuspended in a calcium-containing buffer at physiological pH. This method proved to be superior to the classic platelet aggregometry based on platelet-rich plasma (PRP). The McMaster SRA became widely recognized as a "gold standard" for HIT diagnosis. Several studies demonstrated a higher likelihood of unexplained thrombocytopenia among patients with positive SRA results compared to those with positive ELISA results only or control subjects with negative results. During the SRA, the washing step releases sufficient platelet factor 4 (PF4) to allow activation by HIT sera. To enhance the sensitivity of the SRA, platelets from donors known to react well to HIT sera (referred to as "pedigree" donors) are used, and "weak-positive" control sera are included in each experiment to ensure adequate platelet reactivity (Warkentin 2016). In Australia, the only validated functional assay is the serotonin release assay (SRA), which is batch tested and is only available through the Prince of Wales (POW) laboratory in Sydney, New South Wales. It is unsuitable for STAT or urgent testing (Favaloro 2018).

2. **The heparin-induced platelet activation (HIPA)** test which also employs washed donor platelets, is similar to the SRA. The primary difference lies in the platelet activation endpoint (Warkentin *et al* 2015) where the lag time to aggregation in each reaction well is visually observed. The test does not require radioisotope use (Warkentin *et al* 2015). In the SRA test a single previously identified responsive donor is used but in the HIPA test, platelets from (typically) four previously untested donors are used. Selleng *et al* (2015) suggested that the HIPA may produce platelet aggregation in some samples that test negative in the SRA, indicating a potential for false-positive results with HIPA. The HIPA assay is typically employed in North America and where SRA is otherwise unavailable. It is particularly well suited to sites that have access to platelet donations (i.e. blood banks) (Selleng *et al* 2015).

Diagnostic laboratory challenges

The lack of a gold standard diagnostic tool is one of the primary challenges in recognizing pathological HIT cases. Clinical suspicion, a decline in platelet count, and a positive HIPA or SRA for HIT antibodies are now considered standard diagnostic criteria for HIT. These tests need specialized laboratories and qualified technical staff, and they have limitations including false-positive and false-negative findings. There is no universal agreement on the best cutoff values or positive thresholds, and the interpretation of these tests might be challenging.

Detecting clinically significant HIT is challenging due to the fact that only a small fraction of anti-PF4/polyanion antibodies produced in response to heparin are pathogenic and capable of causing platelet activation, which is a hallmark of HIT (Warkentin 2020).

Thrombocytopenia is a common occurrence in hospitalized patients treated with heparin, so testing for HIT antibodies is frequently performed. Distinguishing between pathogenic and nonpathogenic antibodies is crucial. Platelet activation tests such as the SRA and HIPA can detect antibodies that have platelet-activating properties, which are strongly correlated with pathogenicity (Warkentin 2020). Immunoassays are nonetheless typically used as the initial test for HIT. Clinicians who have access to rapid, on-demand HIT immunoassays, such as particle gel immunoassay, latex immunoturbidimetric assay, and chemiluminescent immunoassay, can adopt semiquantitative interpretation methods (Warkentin 2020). For example, a strong-positive immunoassay result or a combination of positivity in two immunoassays may suggest a higher likelihood of detecting platelet-activating antibodies and, consequently, support a HIT diagnosis. Given that a (false) negative platelet activation assay may occur, the recent identification of SRA-negative HIT underscores the importance of semiquantitative interpretation of immunoassays. Clinicians should therefore pay careful attention to immunoassay reactivity as a possible clue to HIT, even in the absence of platelet activation (Warkentin 2020).

4T Score System

Clinical probability scores are used to determine the likelihood of a patient's thrombocytopenia being caused by pathogenic platelet-activating HIT antibodies prior to testing. Given the common occurrence of thrombocytopenia and non-pathogenic HIT antibodies in hospitalized patients, these scores aid in decision-making both before and after laboratory tests are conducted. The 4T score is the most used clinical probability score (Joseph *et al* 2019). The 4T score is an abbreviated form of Thrombocytopenia, Timing of platelet count fall, Thrombosis and likelihood of oTher causes of thrombocytopenia (Table 2). Each parameter is assigned a score of 0, 1, or 2, based on specific criteria, with the results tabulated resulting in a total score ranging from 0 to 8 (Hvas *et al* 2021).

Low 4T score (0-3) indicate a low probability of HIT and has a significant negative predictive value, thus making it an effective tool for ruling out HIT diagnosis. Pouplard *et al* (2007) demonstrated that a 4T score of $\leq$ 3 was found to have a significant negative predictive value for HIT (0.998; 95% Cl, 0.970-1.000) (Pouplard *et al* 2007). The 4T score does however have a few limitations that should be recognized and missing information may lead to false low 4T score. The combined high (6-8) and intermediate (4,5) scores have a poor positive predictive value since other conditions than HIT can yield similar findings. According to the study by Cuker *et al* (2012), the combined high and intermediate the probability scores had a positive predictive value of 0.22 (0.15-0.31). Nevertheless, high (6-8) and the intermediate (4-5) scores would provide a basis for laboratory testing (Cuker *et al* 2012).

While the 4T score system has proven to be helpful in clinical practice, it is important to acknowledge its limitations. The main limitations of the 4T score system include its subjectivity, lack of sensitivity and specificity, and potential for overdiagnosis or underdiagnosis.

The 4T score system relies on subjective clinical judgment when evaluating the four key parameters: Thrombocytopenia, Timing of platelet count fall, Thrombosis or other sequelae, and the presence of oTher causes of thrombocytopenia. Different clinicians may interpret these parameters differently, leading to potential variability in scoring and subsequent diagnostic decisions.

1. The 4T score system was developed to aid in clinical decision-making, but it is not a definitive diagnostic tool. It has limited sensitivity and specificity, meaning it may not accurately identify all cases of HIT or differentiate them from other causes of thrombocytopenia. False-positive or false-negative results are possible, and additional confirmatory tests may be required.

Table 2*. The 4T score according to Warkentin (2003) is a guideline for determining the likelihood of HIT before laboratory testing.*

Score	2	1	0
Thrombocytopenia	> 50% decrease or platelet nadir $\geq$ 20 x 10^9/L.	30- 50% decrease or platelet nadir 10-20x10^9/L.	< 30% decrease or platelet nadir < 10 x 10^9/L.
Timing	Days 5-10 of clear onset or platelet declining $\leq$ 1 day (previous heparin exposure within 30 days).	Unclear, but consistent with days 5-10 declining of platelets (e.g. missing platelet counts) or onset of thrombocytopenia after day 10.	Early onset of platelet declining (without recent heparin use).
Thrombosis	Skin necrosis, new thrombosis, and a sudden systemic response to a heparin bolus.	Erythematous skin lesions, recurring or progressive thrombosis, and suspected yet unconfirmed thrombosis.	None
oTher cause for low platelets	There is no other apparent explanation for the platelet count declining.	Possible	Definite

4T score rating: 6-8 = high; 4-5 = intermediate; 0-3 = low (Pouplard *et al* 2007).

2. The subjective nature of the 4T score system and its reliance on clinical judgment can contribute to both overdiagnosis and underdiagnosis of HIT Overdiagnosis may lead to unnecessary cessation of heparin therapy or initiation of alternative anticoagulation, which carries its own risks. On the other hand, underdiagnosis may result in continued exposure to heparin, potentially leading to serious complications (Cuker *et al* 2012).

It is important to note that the limitations of the 4T score system have been recognized by the medical community. Efforts are underway to improve diagnostic accuracy by incorporating laboratory tests, such as platelet activation assays or immunoassays, to complement the clinical assessment. These tests can provide additional information to support or refute the diagnosis of HIT.

The 4T score is also mainly used as a clinical tool to guide therapy while anticipating the results of laboratory tests and to support decision-making when those results are made available. The 4T score assists doctors in estimating the probability of HIT and can assist in initiating or ceasing HIT-directed treatments (Cuker *et al* 2012).

Future testing for HIT

HIT remains an important clinical challenge and ongoing research is focused on improving the accuracy and efficiency of HIT screening tests. One area of development in the future testing of HIT screen is the exploration of alternative biomarkers and novel assay techniques.

Studies have investigated the potential use of new biomarkers such as PF4/polyanion complexes and anti-PF4/H antibody isotypes other than IgG, such as IgA, IgM, HIT diagnosis (Warkentin 2019).

Advancement in assay techniques is being further explored. For instance, point-of-care testing (PoCT) devices and rapid tests are being developed to provide immediate results at the patient's bedside, enabling timely decision-making (Cuker *et al* 2018). Additionally advancements in laboratory based assays, such as improved ELISAs, CLIAs and flow cytometry-based assays, are being investigated to enhance the accuracy and efficiency of HIT screening (McKenzie 2022).

It is important to note that the field of HIT screening is rapidly evolving and ongoing research is needed to validate these future testing approaches and determine their clinical utility.

Conclusions

Currently, screening methods for HIT encompass a combination of clinical assessment and laboratory testing. The 4T score system is commonly employed as a clinical tool to evaluate the probability of HIT; however it is not without limitations. The subjective nature of the 4T score system introduces variability in scoring and subsequent diagnostic decisions, and it may not possess the desired sensitivity and specificity. This can result in both overdiagnosis and underdiagnosis of HIT, leading to potential adverse consequences.

To address these limitations and enhance diagnostic accuracy, future testing methods are being explored in the context of HIT screening. In Australia, the implementation of laboratory assays such as platelet activation assays or immunoassays holds promise. The AcuStar has a high specificity and sensitivity but it also has limitations including that it is only available in metro areas, it is an expensive test and requires specific instrumentation and kits. Other screening tests such as ELISA are only available in selected laboratories and only performed in batches. These tests however, can still provide additional objective information to complement the clinical assessment, aiding in the identification and differentiation of HIT from other causes of thrombocytopenia. The integration of such tests into the HIT screening process has the potential to improve diagnostic precision, reduce unnecessary treatment modifications, and minimize the risk of complications associated with misdiagnosis.

It is important for clinicians and scientists in Australia to be aware of the limitations of current HIT screening methods and to stay updated on advancements in diagnostic techniques. Continued research and development in this field are crucial for refining the screening process and ensuring optimal patient care. By combining clinical judgment with laboratory testing, clinicians can strive for more accurate and reliable HIT diagnosis, leading to improved patient outcomes. There are plenty of opportunities for improvement within all laboratories for HIT screening methods.

It remains important to consult current guidelines, protocols, and recommendations from relevant Australian healthcare authorities for the most up-to-date information on HIT screening methods and limitations.

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References

Arepally GM 2017. Heparin-Induced Thrombocytopenia. Blood, 129(21), 2864-2872. https://doi.org/10.1182/blood-2016-11-709873.

Arepally GM, Ortel TL 2006. Clinical Practice. Heparin-Induced Thrombocytopenia. *The New England Journal of Medicine, 355*(8), 809.

Carpenè G, Negrini D, Lippi G, Favaloro EJ, Montagnana M 2022. Heparin: The Journey from Parenteral Agent to Nasal Delivery. *Seminars in Thrombosis and Hemostasis, 48*(8), 949-954. https://doi.org/10.1055/s-0042-1749395.

Cuker A, Arepally GM, Chong BH, Cines DB, Greinacher A, Gruel Y, Linkins LA, Rodner SB, Selleng S, Warkentin TE, Wex A, Mustafa RA, Morgan RL, Santesso N 2018. American Society of Hematology 2018 Guidelines for Management of Venous Thromboembolism: Heparin-Induced Thrombocytopenia. *Blood advances, 2*(22), 3360-3392. https://doi.org/10.1182/bloodadvances.2018024489.

Cuker A, Gimotty PA, Crowther MA, Warkentin TE 2012. Predictive Value of the 4ts Scoring System for Heparin-Induced Thrombocytopenia: A Systematic Review and Meta-Analysis. *Blood, 120*(20), 4160-4167. https://doi.org/10.1182/blood-2012-07-443051.

Favaloro EJ 2018. Laboratory Tests for Identification or Exclusion of Heparin Induced Thrombocytopenia: Hit or Miss? *American Journal of Hematology, 93*(2), 308-314. https://doi.org/10.1002/ajh.24979.

Gardiner EE, Andrews RK, Cuker A 2014. Diagnostic Assays for Heparin-Induced Thrombocytopenia. *British Journal of Haematology, 166*(5), 631-633. https://doi.org/10.1111/bjh.12940.

Greinacher A 2015. Heparin-Induced Thrombocytopenia Reply. *The New England Journal of Medicine, 373*(19), 1883-1884.

Greinacher A, Warkentin TE 2006. Recognition, Treatment, and Prevention of Heparin-Induced Thrombocytopenia: Review and Update. *Thrombosis Research, 118*(2), 165-176. https://doi.org/10.1016/j.thromres.2005.07.012.

Hvas AM, Favaloro EJ, Hellfritzsch M 2021. Heparin-Induced Thrombocytopenia: Pathophysiology, Diagnosis and Treatment. Expert Review of Hematology, 14(4), 335-346. https://doi.org/10.1080/17474086.2021.1905512.

Joseph J, Rabbolini D, Enjeti AK, Favaloro E, Kopp MC, McRae S, Pasalic L, Tan C W, Ward CM, Chong BH 2019. Diagnosis and Management of Heparin-Induced Thrombocytopenia: A Consensus Statement from the Thrombosis and Haemostasis Society of Australia and New Zealand Hit Writing Group. *Medical Journal of Australia, 210*(11), 509-516. https://doi.org/10.5694/mja2.50213.

Kumar N, Uppal V, Ahluwalia J, Malhotra P, Varma N, Jain A 2019. Evaluation of Stic Expert(®) Hit Kit and Its Comparison with Id-Pagia™ Test in Suspected Heparin-Induced Thrombocytopenia. *Indian Journal Hematology Blood Transfusion, 35*(1), 155-160. https://doi.org/10.1007/s12288-018-0996-z.

Linkins LA, Dans AL, Moores LK, Bona R, Davidson BL, Schulman S, Crowther M 2012. Treatment and Prevention of Heparin-Induced Thrombocytopenia: Antithrombotic Therapy and Prevention of Thrombosis, 9th Ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest, 141*(2), e495S-e530S. https://doi.org/10.1378/chest.11-2303.

Lo GK, Juhl D, Warkentin TE, Sigouin CS, Eichler P, Greinacher A 2006. Evaluation of Pretest Clinical Score (4 T's) for the Diagnosis of Heparin-Induced Thrombocytopenia in Two Clinical Settings. *Journal of Thrombosis and Haemostasis, 4*(4), 759-765. https://doi.org/10.1111/j.1538-7836.2006.01787.x.

Magnani HN 1993. Heparin-Induced Thrombocytopenia (Hit): An Overview of 230 Patients Treated with Orgaran (Org 10172). *Thrombosis Haemostasis, 70*(4), 554-561.

McKenzie SE 2022. A Vision for a Quicker Definitive Diagnosis of Hit. *Blood, 140*(25), 2657-2658. https://doi.org/10.1182/blood.2022018158.

Nagler M, Bachmann LM, ten Cate H, ten Cate-Hoek A 2016. Diagnostic Value of Immunoassays for Heparin-Induced Thrombocytopenia: A Systematic Review and Meta-Analysis. *Blood, 127*(5), 546-557. https://doi.org/10.1182/blood-2015-07-661215.

Pouplard C, Gueret P, Fouassier M, Ternisien C, Trossaert M, Régina S, Gruel Y 2007. Prospective Evaluation of the '4ts' Score and Particle Gel Immunoassay Specific to Heparin/Pf4 for the Diagnosis of Heparin-Induced Thrombocytopenia. *Journal of Thrombosis and Haemostasis, 5*(7), 1373-1379. https://doi.org/10.1111/j.1538-7836.2007.02524.x.

Refaai MA, Conley G, Ortel TL, Francis J L 2019. Evaluation of a Rapid and Automated Heparin-Induced Thrombocytopenia Immunoassay. *International Journal of Laboratory Hematology, 41*(4), 478-484. https://doi.org/10.1111/ijlh.13029.

Sachan D, Gupta N, Chaudhary R 2013. Rapid Diagnosis of Heparin-Induced Thrombocytopenia Using a Particle Gel Immunoassay in at-Risk Cardiac Surgery Patients. *Asian Journal Transfusion Science, 7*(1), 92-93. https://doi.org/10.4103/0973-6247.106765.

Sahu KK, Jindal V, Anderson J, Siddiqui AD, Jaiyesimi IA 2020. Current Perspectives on Diagnostic Assays and Anti-Pf4 Antibodies for the Diagnosis of Heparin-Induced Thrombocytopenia. *Journal Blood Medicine, 11*, 267-277. https://doi.org/10.2147/jbm.S232648.

Selleng S, Selleng K, Friesecke S, Gründling M, Kuhn SO, Raschke R, Heidecke OJ, Hinz C, Hron G, Warkentin TE, Greinacher A 2015. Prevalence and Clinical Implications of Anti-Pf4/Heparin Antibodies in Intensive Care Patients: A Prospective Observational Study. *Journal Thrombosis Thrombolysis, 39*(1), 60-67. https://doi.org/10.1007/s11239-014-1105-2.

Warkentin TE 2003. Heparin-Induced Thrombocytopenia: Pathogenesis and Management. *British Journal of Haematology, 121*(4), 535-555. https://doi.org/10.1046/j.1365-2141.2003.04334.x.

Warkentin TE 2004. Heparin-Induced Thrombocytopenia: Diagnosis and Management. *Circulation (New York, N.Y.), 110*(18), e454-458. https://doi.org/10.1161/01.CIR.0000147537.72829.1B.

Warkentin TE 2016. Platelet Activation Testing for Heparin-Induced Thrombocytopenia Antibodies: A Problem That Needs Fixing? *Chest, 150*(3), 478-480. https://doi.org/10.1016/j.chest.2016.04.001.

Warkentin TE 2019. Laboratory Diagnosis of Heparin-Induced Thrombocytopenia. *International Journal of Laboratory Hematology, 41*(1), 15-25. https://doi.org/10.1111/ijlh.12993.

Warkentin TE 2020. Challenges in Detecting Clinically Relevant Heparin-Induced Thrombocytopenia Antibodies. *Hämostaseologie, 40*(4), 472-484. https://doi.org/10.1055/a-1223-3329.

Warkentin TE, Arnold DM, Nazy I, Kelton JG 2015. The Platelet Serotonin-Release Assay. *American Journal of Hematology, 90*(6), 564-572. https://doi.org/https://doi.org/10.1002/ajh.24006.

Warkentin TE, Greinacher A 2004. Heparin-Induced Thrombocytopenia: Recognition, Treatment, and Prevention - the Seventh Accp Conference on Antithrombotic and Thrombolytic Therapy. *Chest, 126*(3), 311S-337S. https://doi.org/10.1378/chest.126.3_suppl.311S.

Warkentin TE, Greinacher A, Koster A, Lincoff AM 2008. Treatment and Prevention of Heparin-Induced Thrombocytopenia: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest, 133*(6), 340S-380S. https://doi.org/10.1378/chest.08-0677.

Warkentin TE, Sheppard JAI, Horsewood P, Simpson PJ, Moore JC, Kelton JG 2000. Impact of the Patient Population on the Risk for Heparin-Induced Thrombocytopenia. *Blood, 96*(5), 1703-1708. https://doi.org/10.1182/blood.V96.5.1703.

Warkentin TE, Sheppard JAI, Linkins L.-A, Arnold DM, Nazy I 2017. Performance Characteristics of an Automated Latex Immunoturbidimetric Assay [Hemosil® Hit-Ab(Pf4-H)] for the Diagnosis of Immune Heparin-Induced Thrombocytopenia. *Thrombosis Research, 153,* 108-117. https://doi.org/10.1016/j.thromres.2017.03.010.

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Journal-based CPD No. 96
Page 1 of 2

Questions relating to the article *'The impact of COVID-19 on blood transfusion services at Colonial War Memorial Hospital, Suva, Fiji'* at page 5 of this issue.

1.	Blood donations showed a decline each year, 8.8% decline was noted between the years 2019 to 2020 and a 4.69% decline between the 2020 and 2021 period.	True/False
2.	Blood donations were critically low between April 2020 to June 2020.	True/False
3.	Between January 2019 and March 2022, 86% of the blood donations were male.	True/False
4.	The highest higher number of blood donations were between the ages of 16-44y.	True/False
5.	A decline in all blood component usage was noted each year due to a decrease in elective surgeries.	True/False
6.	Donor age eligibility criteria in Fiji has changed from 18-60y to 16-65y.	True/False
7.	The highest deferral rate was from physical causes with high haemoglobin and high blood pressure as the commonest causes.	True/False
8.	Platelet usage did not show a significant decline during the pandemic period 2020-2021.	True/False
9.	During the pandemic, high blood pressure was seen to increase by 50% from 2020 to 2021.	True/False
10.	It is recommended that the donor age category 16-25y needs to be targeted as a significant rise was shown in this age bracket.	True/False

Name: ____

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Journal-based CPD No. 97
Page 2 of 2

Questions relating to the article '*Strategies for isolating microbiota from faecal samples: are all gut microorganisms potentially cultivable?*' at page 24 of this issue.

1.	90% of the gut microbiota is composed of four major microbial phyla: Firmicutes, Bacteroides, Proteobacteria and Actinobacteria.	True/False
2.	Challenges in growing some gut bacteria include the requirement for factors produced by other microbes, interspecies competition/inhibition, dormancy and slow growth.	True/False
3.	The gut microbiota consists mostly of aerobic bacteria.	True/False
4.	Culturomics increases the chances of growing fastidious bacteria by using a wide range of media and growth temperatures.	True/False
5.	MALDI-TOF MS mass spectrometry and 16S rRNA gene sequencing are used to identify organisms.	True/False
6.	Less commonly used media include Brain Heart Infusion Agar & Columbia Agar.	True/False
7.	The single biggest disadvantage to bacterial culture is the length of time required to obtain a pure culture.	True/False
8.	The addition of antibiotics added to culture media can significantly modify the composition of the bacterial population and selectively isolate target species.	True/False
9.	Culturing gut bacteria is challenging and if used as the primary tool for diagnosing GI infections may lead to clinically significant bacteria being missed in faecal samples.	True/False
10.	The most widely used growth temperature is 37°C.	True/False

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Changes to APACE due to COVID-19 pandemic UPDATE

An APACE Certificate is usually awarded on attaining 100 CEU credits within a two year period.

As webinars and online conferences, meetings and workshops are all interactive, it was considered that this is the same as attending in person, therefore the same number of points will be awarded for attendance either virtually or face-to-face. This should enable more members to attend as no travelling time, costs and in some cases the online attendance with be without cost to the attendee.

Therefore, the extended time frame due to Covid-19 will no longer apply.

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AUSTRALIAN JOURNAL OF MEDICAL SCIENCE

Instructions to authors

The following instructions are based on the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals", also known as the Declaration of Vancouver, and on the *Australian Government Style manual: for authors, editors and printers*, 6th edition, 2002. URLs were correct on September 29th, 2008.

Manuscripts that do not fully comply with the following 'Instructions to Authors' may be returned for revision before they are considered for publication.

The *Australian Journal of Medical Science (AJMS)* will consider for publication any paper relevant to the field of Medical Science. Disciplines include blood banking, clinical biochemistry, haematology, histopathology, immunology, microbiology and molecular biology. Areas of general interest to medical laboratory scientists, including toxicology, epidemiology, public and community health, and professional and management issues will also be considered.

Papers published in the *AJMS* are in the form of:

- Review Articles
- Original Articles
- Brief Communications
- Technical Notes
- Case Studies
- Letters to the Editor
- Book Reviews

Articles submitted for publication are understood to be offered only to the *AJMS* and those accepted become the property of the *AJMS*.

All individuals listed as authors must have made a substantial contribution to the conception and design of the study, the acquisition of data or the analysis and interpretation of data; the drafting of the article or revising it critically for important intellectual content; and final approval of the version to be published. The corresponding author must take responsibility for obtaining permission from all the authors for the submission of any version of the manuscript and for any changes in authorship.

When the manuscript is submitted the authors must disclose any potential conflict of interest and/or commercial support.

Requirements & preparation of manuscripts

General

Articles should be submitted in electronic format to programs@aims.org.au. If an article is too large to be submitted by email, it should be submitted on an or USB stick.

Number pages consecutively commencing with the title page.

Arrange the article in the following sequence:

- Title page
- Abstract and key words
- Main Text
- Acknowledgements
- References
- Tables - each table, complete with title and footnotes, on a separate page
- Legends for illustrations.

Authors should ensure that their manuscript communicates their ideas and concepts simply and clearly so that the article is easily read and understood. Authors are strongly recommended to refer to the recommendations on reporting standards as outlined in the statements and checklists of the CONSORT group (see: http://www.consort-statement.org/) and similar groups such as STARD (see: http://www.stard-statement.org/). The principles outlined in these standards may be used as general guidelines and not just as applied to clinical trials and diagnostic studies.

Title page

The title of the article should not exceed three lines (40 characters per line), including punctuation and spacing. All authors must be identified on the title page (e.g. William Smith, Susan Yeo, …"). Where applicable, the title page should also include the name of the institution with which each author is affiliated and to which the work should be attributed. In the case of multiple authors, the name, postal address, email address, telephone and facsimile number of the author responsible for correspondence relating to the manuscript should be indicated.

Abstract & keywords

The abstract should be approximately 150 words and should make sense when read alone or in conjunction with the article. The abstract should be a concise overview that describes the important details of the article including the purpose of the study/investigation, basic procedures (study subjects/experimental animals/observational and analytic methods) and the results and principal conclusions. New and important aspects of the work and its implications may also be included. References should not be included.

Three to ten keywords may be listed. Authors are advised to comply with the terms from the Medical Subject Headings (MeSH) list from Index Medicus (see http://www.nlm.nih.gov/mesh/). Keywords should be given below the Abstract.

Text

The style of writing should conform to acceptable English usage. Do not use slang, medical jargon or unnecessary abbreviations. Accepted spelling is the first choice given in the latest edition of the Macquarie Dictionary.

Wherever possible, observational or experimental articles should be divided into sections headed:

- Introduction
- Materials and methods
- Results
- Discussion
- References

For other types of articles such as commentaries, reports and reviews, use an appropriate format or consult the Editors for guidance. Do not include a separate section for conclusions, these should be given in the discussion.

Introduction

Clearly state the purpose of the article leading the reader from the known to the unknown. Summarise the rationale for the study and state the question to be answered as appropriate. Give only strictly pertinent references, and do not review the subject extensively.

Materials & methods

Present the materials and methods in a logical sequence. Describe the selection of the observational or experimental subjects (patients or experimental animals, including controls) clearly. Notification of ethics approval must be given where relevant. Identify the methods, apparatus and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods. Adequately describe new or substantially modified methods. Identify precisely all drugs and chemicals used, including generic name(s), dosage(s), and route(s) of administration. Do not identify patients or hospitals without consent.

Results

Present the results in the same sequence as given in the Materials and methods; use tables and illustrations where these will help the reader understand the work being presented. Do not repeat in the text all the data in the tables or illustrations.

Discussion

Indicate the new and important aspects of the study and emphasise the conclusions that follow. Do not repeat in detail data given in the Results section and do not add new data. Include in the Discussion the implications of the findings and their limitations and compare the observations to other relevant studies. Recommendations may be included if appropriate. Link the conclusions with the goals of the study and answer the experimental question stated in the Introduction. However, avoid unqualified statements and conclusions not completely supported by your data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such.

Acknowledgements

Acknowledge individuals who have made substantial contributions to the study including technical work and financial support. Authors are responsible for obtaining consent from all the individuals acknowledged by name as inclusion may be interpreted as an endorsement of the article's contents.

References

The AJMS uses a modified Harvard System (author-date system).

Throughout the body of the manuscript cite the author/s name and the publication year in parentheses as in the following examples:

(i) Research in this area (Jones 1999) …

(ii) It has been successfully demonstrated that (Smith and Brown 1981; Auteur 1995; Scienziato et al 2007).

(iii) Following further investigation, Wetenschapper (2002 highlighted the difficulties inherent in…

Where there are three or more authors, acknowledge only the first author, e.g. (Smith et al 2007). For two authors the following style should be used: (Smith and Brown 2007).

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Examples of the correct form for references are given below:

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Online documents:

National Center for Biotechnology Information. OMIM: online Mendelian inheritance in man. http://www.ncbi.nlm.nih.gov/omim. Accessed February 25, 2007.

Tables

Number tables consecutively with Arabic numerals and supply a brief title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in headings. Explain in footnotes all non-standard abbreviations used in each table.

For footnotes, use the following symbols in this sequence:

* † ‡ § ¶ ** ††

In preparing tables, consideration should be given to the page width of the Australian Journal of Medical Science. All tables should be prepared for publication vertically. In the text, cite each table in consecutive order, and mark in the margin of the text its approximate location.

If data from another published or unpublished source is used, written permission must be obtained and a copy must accompany the manuscript.

Illustrations

Colour illustrations may be submitted on a USB stick. Images should be scanned at a minimum of 300 dpi.

When plotting points, the following symbols are preferred:

In most instances, figures will be reduced to one column in width. All letters and numbers should be drawn to be at least 1.5 mm high after reduction, symbols at least 1.0 mm. Titles for illustrations belong in the legends for illustrations and not on the illustrations themselves.

Photomicrographs must have internal scale markers and the magnification must be stated. Symbols, arrows, or letters used in the photomicrographs should contrast with the background.

Cite each figure in the text in consecutive order, e.g. "Figure 1 illustrates ..." or "... as shown (Figure 2)". If a figure has been published, acknowledge the original source and submit with the manuscript written permission from the copyright holder to reproduce the material. Permission is required, regardless of authorship or publisher, except for documents in the public domain.

Legends for illustrations

When symbols, arrows, numbers, or letters are used to identify parts of illustrations, identify and explain each one in the legends. The figure legend must contain a boldface (a) name ("Figure" + arabic figure number) and (b) substantive title.

Abbreviations

Use only standard abbreviations (see list of commonly used abbreviations).

Avoid abbreviations in the title. The full term for which an abbreviation stands must precede its first use in the text unless it is a standard abbreviation for a unit of measurement.

Report measurements in the units in which the measurements were made. In most countries the International System of Units (SI) is standard.

Commonly used abbreviations

Abbreviation or Symbol	Standard Units of Measurement
g	gram
g	gravity
Hz	hertz
h	hour
IU	international unit
K	kelvin
kg	kilogram
L	liter, litre
m	meter, metre
min	min
M	molar
mL	millilitre
mol	mole
N	newton
nm	nanometre
p	probability
rpm	revolutions per min
s	second
wk	week
yr	year

Additional information

The following are useful sources of information. The first two publications are used by the AJMS as standard references.

Style Manual Committee. Council of Biology Editors. *Scientific style and format: the CBE manual for authors, editors, and publishers.* 6th ed. Cambridge University Press, 1994.

Style manual for authors, editors and printers. 6th ed. John Wiley & Sons Australia Ltd, 2002.

O'Connor M, Woodford FP. *Writing scientific papers in English: an ELSE-Ciba Foundation guide for authors.* Amsterdam, Oxford, New York: Elsevier-Excerpta Medica, 1975.

Day RA. *How to write and publish a scientific paper.* Philadelphia, Institute for Scientific Information Press, 1979.

Zeiger M. *Essentials of writing biomedical research papers*. 2nd ed. New York, McGraw-Hill, 2000.

Matthews JR, Matthews RW. *Successful scientific writing: a step-by-step guide for the biological and medical sciences*. 3rd ed. Cambridge, Cambridge University Press, 2007 [Also available in eBook format.]

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Changes to Certification arrangements for the Medical Laboratory Science Profession

From April 2023, the Australian Council for the Certification of the Medical Laboratory Scientific Workforce (CMLS) Board are no longer accepting applications for certifications directly. Instead, professional bodies operating CMLS approved CPD schemes will be able to issue certification on behalf of the Council for their members who meet the requirements for certification as detailed on the CMLS website.

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