

Typography and text design  
for  
science editors

## Designing effective documents

A communication is effective if its ‘wording, structure, and design are so clear that the intended audience can easily find what they need, understand what they find, and use that information’.

From definition adopted by International Plain Language Working Group

## Blunder at the Oscar awards ceremony

It was the greatest mistake in Academy Awards history. *Moonlight* . . . won over *La La Land* . . . but *Moonlight*'s win was overshadowed by a massive blunder which saw Faye Dunaway announce *La La Land* as the Best Picture.

The  
OSCAR<sup>®</sup>S.

**"MOONLIGHT"**

**ADELE ROMANSKI, DEDE GARDNER  
AND JEREMY KLEINER, PRODUCERS**

*Best Picture*

BEST PICTURE

**"MOONLIGHT"**

Adele Romanski, Dede Gardner  
and Jeremy Kleiner, Producers

The  
OSCARS<sup>®</sup>

# Good practice: Abstract in larger font

## Original research

### Accessing care in multidisciplinary pain treatment facilities continues to be a challenge in Canada

Manon Choinière,<sup>1,2</sup> Philip Peng,<sup>3,4</sup> Ian Gilron,<sup>5,6</sup> Norman Buckley,<sup>7,8</sup> Owen Williamson,<sup>9,10</sup> Audree Janelle-Montcalm,<sup>2</sup> Krista Baerg,<sup>11,12</sup> Aline Boulanger,<sup>1,13</sup> Tania Di Renna,<sup>14,15</sup> Gordon Allen Finley,<sup>16,17</sup> Howard Intrater,<sup>18,19</sup> Brenda Lau,<sup>20,21</sup> John Pereira<sup>22,23</sup>

For numbered affiliations see end of article.

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#### ABSTRACT

**Background** Multidisciplinary pain treatment facilities (MPTFs) are considered the optimal settings for the management of chronic pain (CP). This study aimed (1) to determine the distribution of MPTFs across Canada, (2) to document time to access and types of services, and (3) to compare the results to those obtained in 2005–2006. **Methods** This cross-sectional study used the same MPTF definition as in 2005–2006—that is, a clinic staffed with professionals from a minimum of three different disciplines (including at least one medical specialty) and whose services were integrated within the facility. A comprehensive search strategy was used to identify existing MPTFs across Canada. Administrative leads at each MPTF were invited to complete an online questionnaire regarding their facilities.

**Results** Questionnaires were completed by 104 MPTFs (response rate 79.4%). Few changes were observed in the distribution of MPTFs across Canada compared with 17 years ago. Most (91.3%) are concentrated in large urban cities. Prince Edward Island and the Territories still lack MPTFs. The number of pediatric-only MPTFs has nearly doubled but remains small (n=9). The median wait time for a first appointment in publicly funded MPTFs is about the same as 17 years ago (5.5 vs 6 months). Small but positive changes were also observed.

**Conclusion** Accessibility to public MPTFs continues to be limited in Canada, resulting in lengthy wait times for a first appointment. Community-based MPTFs and virtual care initiatives to distribute pain services into regional and remote communities are needed to provide patients with CP with optimal care.

#### INTRODUCTION

About one in five Canadians reports from chronic pain (CP).<sup>1–2</sup> Persistent pain has numerous deleterious effects on physical, psychological, and social functioning and thereby contributes deteriorating health-related quality of life.<sup>3–6</sup> While Canadian data are less robust, the estimated total direct health-care costs of CP and its indirect costs related to loss of productivity range between 560 and 635 billion annually in the USA.<sup>7</sup> Applying these estimates to the Canadian population, the total annual costs of CP would exceed 56 billion.<sup>8</sup>

The International Association for the Study of Pain recommends an integrated multimodal care model for the management of CP because it is a complex phenomenon which is multidimensional

in nature.<sup>9–10</sup> In its 2019 report, the Canadian Pain Task Force concluded that pharmacological treatments are most effective when combined with physical, psychological, and self-management techniques within an integrated multidisciplinary pain management plan.<sup>5</sup>

Multidisciplinary pain treatment facilities (MPTFs) exist within tiered pain management networks to provide integrated multimodal care for people living with CP particularly when it is associated with mood and substance use disorders. MPTFs also provide interventional procedures, education, training, research, and support to those who provide care in community or primary care settings.

In 2005–2006, Peng et al found that the median wait time for a first consultation in a Canadian public MPTF was 6 months and could be as long as 5 years.<sup>11</sup> Furthermore, 80% of clinics were concentrated in major urban cities and there were none on Prince Edward Island or in the Territories. Since 2005, the population of Canada has increased 16.9% and aged, with the proportion of those 65 years and older having augmented from 13.1% to 17.5%,<sup>12,13</sup> resulting in a growing demand on MPTF's services. An update of the Canadian MPTF's landscape is also warranted in the context of the opioid crisis and its impact on our healthcare system.

Furthermore, in recent months, the capacity to maintain pain services has been challenged by the impact of the COVID-19.<sup>14</sup>

In laying the foundation for the development and implementation of a Canadian national pain strategy, it is important to review MPTFs in order to inform the development of policy that improves the care of those living with CP and plans for continuity of care in times of crisis such as the COVID-19 pandemic.<sup>14</sup>

The aims of this study were (1) to examine the current distribution of MPTFs in Canada's 10 provinces and 3 territories, (2) to document time to access and types of services, and (3) to compare these results to those obtained in 2005–2006.

#### METHODS

This study used the same overall methods as the ones we used in 2005–2006.<sup>11</sup> A comprehensive search strategy coupled to the administration of a self-reported questionnaire were used to map and

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## Regional Anesthesia and Pain Medicine

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BMJ

Choinière M, et al. *Reg Anesth Pain Med* 2020;45:943–948. doi:10.1136/rapm-2020-101935

943

## Before

### Quis et Eseribh

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# After

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- 2) Agnum quate cum nra quate - Molorem ing ea faccumry nalla comrod do dant wisit, cum dolorem ansequisicng nra et nra vulpate enim ing enim dip otapia nalla consequam ver sim zrtasito et at. Am dolore ea feuguet si ex eros ea autpat, Conne modo od dais nostrud ea facilla.
- 3) Exeratam iliquisim nia doloboret daisi - Utiam quat wis nra zrtasilia ex enim dolobore minim do con hendre tarsand iperitiosed dolorotarnry Agnum quat cum nra quate molorem ing ea faccumry nalla comrod do dant wisit, nam.

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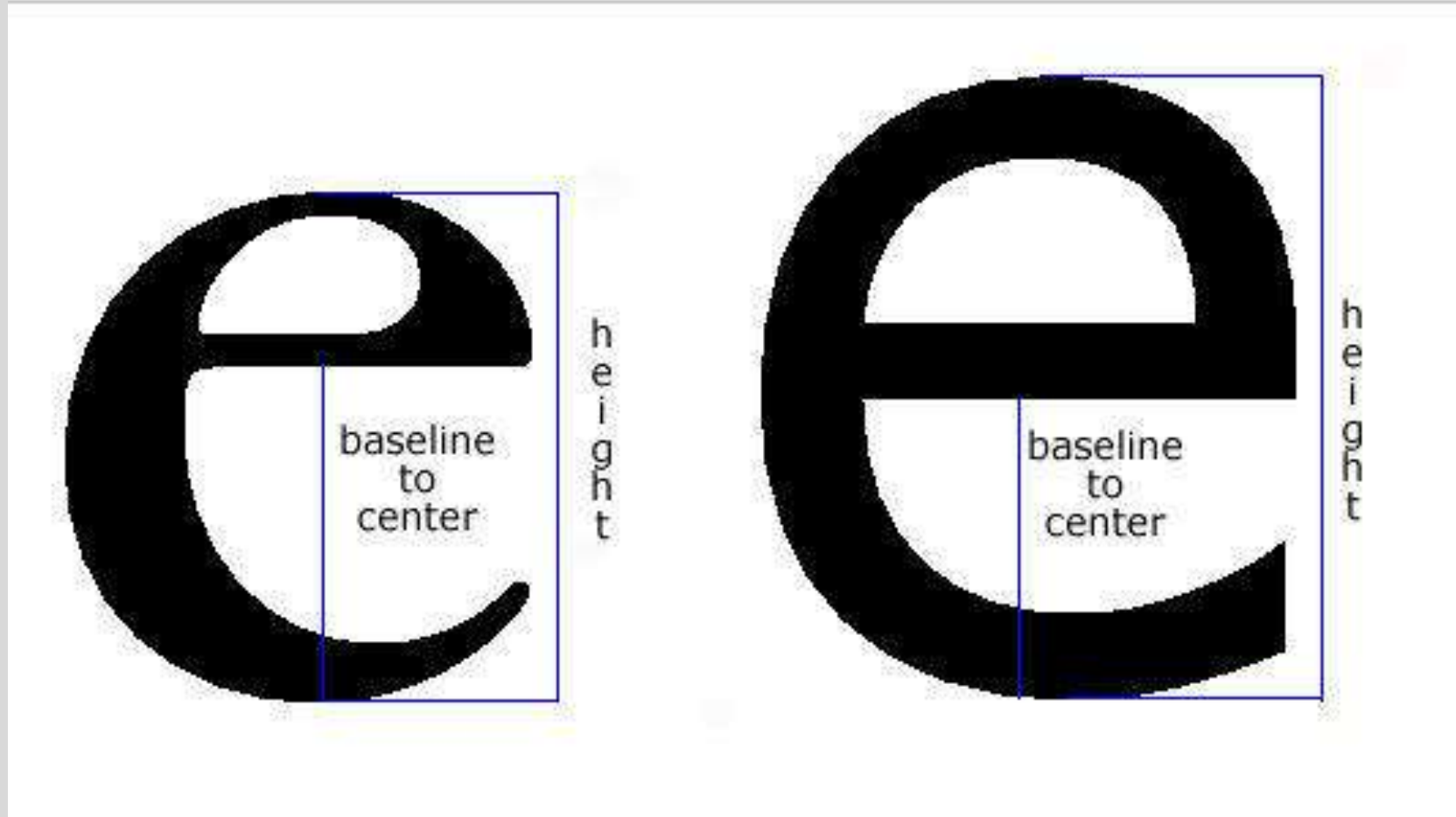
## From font to a published document

- Letters: font (typeface)
- Words, spacing between words
- Lines: length, line endings
- Paragraphs: line spacing, para markers
- Headings: hierarchy (levels of headings)

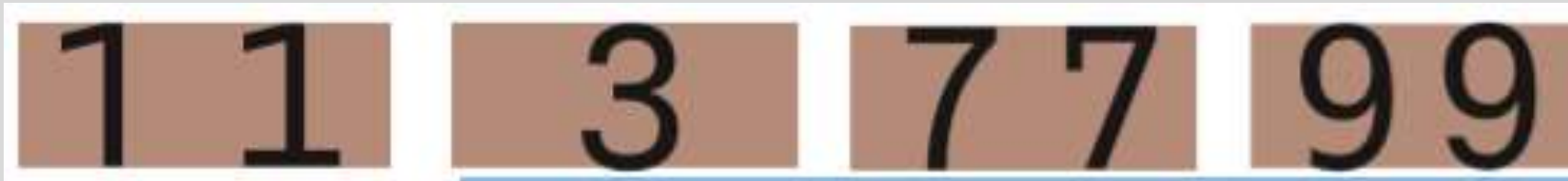
## Examples of research, 1

- Characters shown in random sequence
- Twenty typefaces, 47 characters:  
a-z, 0-9, ÷ = + ? % ± \$ # @ & !
- Each character for 34 ms at 1.5 seconds
- Scored for accuracy
- Small **c** and **e**: Garamond, 10% correct; Verdana, 100% correct

## Legibility of small **C** and **e**



## Examples of research, 2



Top row significantly more legible than bottom row

## Choosing a font, 1

- Required characters and symbols available
- Legible at small sizes
- Clearly distinguishable characters
- Easy availability on most devices and systems

## **Nature journal redesigned, 1**

‘Science sorely needs best practices in visual communication as well as in information design, a mature field with quantitative methods.’

— Kelly Krause, Creative Director, *Nature*

**“The ‘flavour’ of the typeface  
– the feelings it evokes, its  
personality – evolved over  
several months.”**

## Nature journal redesigned, 2

characters. So we have made the sub- and superscript characters larger than standard, and created a Greek alphabet carefully honed to convey scientific meaning rather than typical Greek-language prose – for example, clearly rendering an alpha ( $\alpha$ ) in a shape that looks like a mathematical symbol, so that it is not easily confused with a Latin italic letter *a*. We have also made the italics more slanted so they are more distinct; single italic characters, such as *h* for Planck's

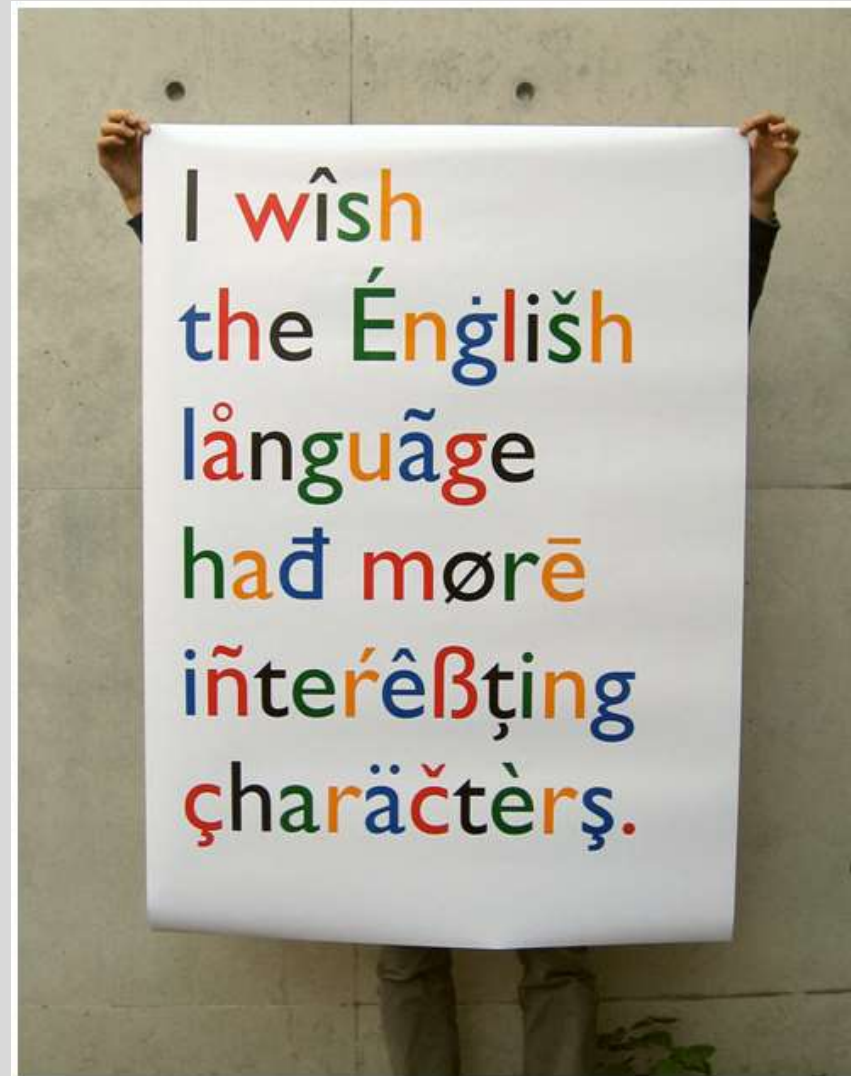
Harding is designed to cope across the disciplines. It boasts an unusually large range of special characters, from triple prime and nabla to a full set of astronomical symbols and the 'click' phonemes found in some African languages.

A key consideration in Harding's overall design is performance on small digital screens. To boost readability in a limited space, it helps to enlarge the main portion of the lower-case letters, while making the ascenders and descenders (as in 'h' and 'g', respectively) smaller. Ultimately, this renders long, complex strings of words easier

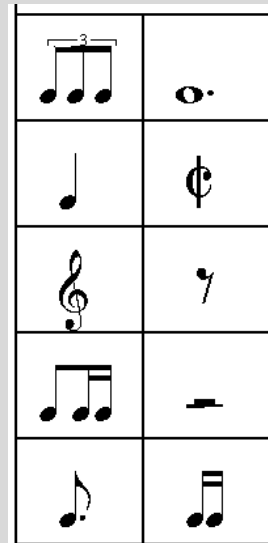
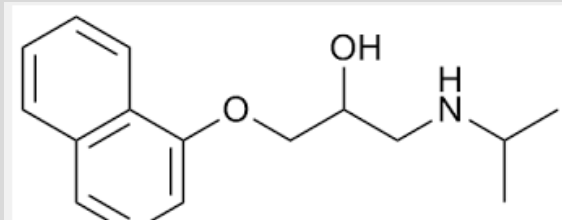
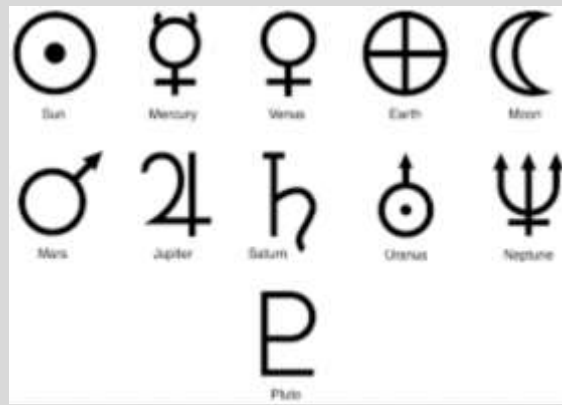




## Required characters, 1



## Required characters, 2



A α	alpha	N ν	nu
B β	beta	Ξ ξ	ksi
Γ γ	gamma	Ο ο	omicron
Δ δ	delta	Π π	pi
E ε	epsilon	Ρ ρ	rho
Z ζ	zeta	Σ σς	sigma
H η	eta	Τ τ	tau
Θ θ	theta	Υ υ	upsilon
I ι	iota	Φ φ	phi
K κ	kappa	X χ	chi
Λ λ	lambda	Ψ ψ	psi
M μ	mu	Ω ω	omega

## Choosing a font, 2

- Appropriate ‘atmosphere’ value: formal, informal, technical, elegant, neutral, funky, . . .

## From highly format to very informal



# Comic Sans



# Use well-designed fonts: I l 1 test

I l 1

Verdana

I l 1

Droid Sans

I l 1

FF Meta

I l 1

National

I l 1

Heinemann

I l 1

Avenir

I l 1

Futura

I l 1

Gotham

I l 1

Benton Sans

I l 1

Helvetica

I l 1

Gill Sans

**The I l 1 Test**

*Not the ultimate legibility  
determiner, but helps*

I l 1

Mostra Nuova

## Fonts for small sizes, 1

- Minuscule, by Thomas Huot-Marchand  
Five versions, optimized for 2–6 points  
6 (Minuscule Six), 5 (Cinq), 4 (Quatre),  
3 (Trois), and 2 (Deux)
- Bell Centennial, by Mathew Carter  
for telephone directories

## Fonts for small sizes, 2

MINUSCULE 4

ag

MINUSCULE 2

ad

MINUSCULE 3

Typographies

MINUSCULE 2

Compactes.



## Fonts for large sizes

- Quarto meant for use at 16 points or larger.

**Myths and Metaphors**

- ClearView for US highway signs: easier to read at long distances and in darkness

Typefaces without serifs and with serifs

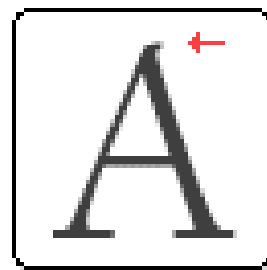
I N D

I N D

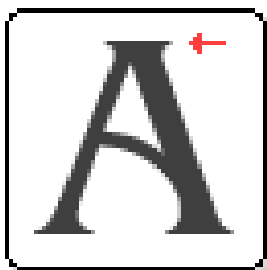
## Apex of capital A



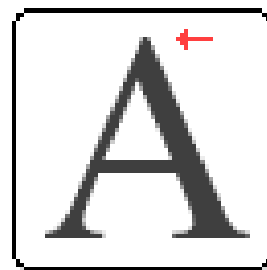
Serif or cusp on left.



Serif or cusp on right.



Serifs both sides.

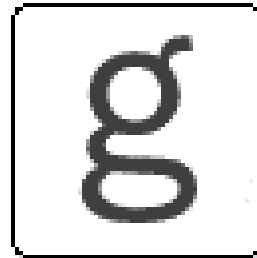


No serifs or cusps.

## Lowercase (small) g



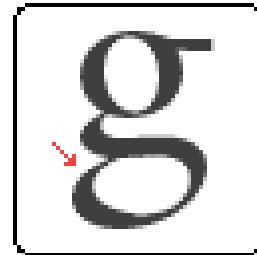
Single-storey (with or without loop).



Double-storey (with or without gap).

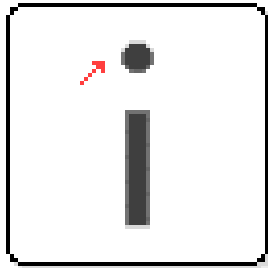


Gap.

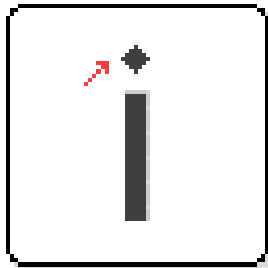


No gap.

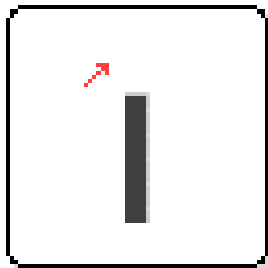
## Dot on small i and j



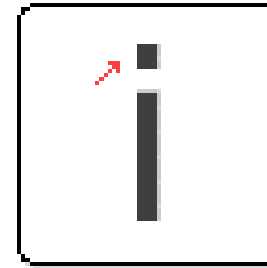
Circular or oval dot.



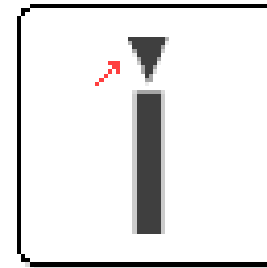
Diamond-shaped dot.



No dot.



Square or rectangular dot.



Triangular dot.

## Avoid spacing lowercase letters

11. Barman, A., Prabhu, P., Narne, V.K., Singh, N.K. & Spoorthi, T. (2017). Low frequency bi-syllabic wordlists in a south-Indian language, Kannada: Development, standardization and validation. **Hearing**, Balance & Communication, 15(1), 38-47.

12. Barman, A., Prabhu, P., Narne, V.K., Thammaiah, S., Singh, N.K., & Gupta, M. (2017). Development and standardization of auditory low-frequency word lists in Hindi. Journal of **Hearing** Science, 6(4), 39-49. doi: 10.17430/899781.

## Spacing between words: even right edge but variable word spacing

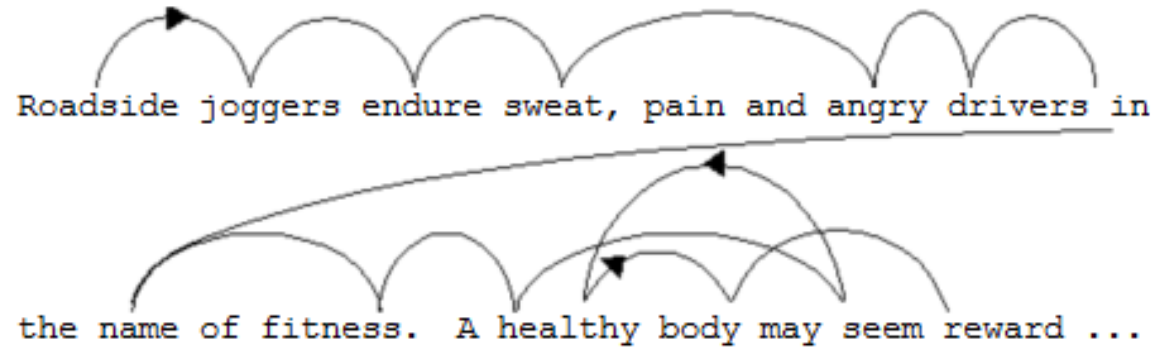
- ⇒ At the very back of the eye is the retina, which is the image-detecting zone. If you look at the retina under a microscope, there is a tangle of cells seemingly forming a jumbled mass that looks somewhat like a net—hence the name retina, from
- ⇒ the Latin rete for ‘net’. These cells in the retina respond to changes of light.

## Spacing between words: uneven right edge but uniform word spacing

At the very back of the eye is the retina, which is the image-detecting zone. If you look at the retina under a microscope, there is a tangle of cells seemingly forming a jumbled mass that looks a little like a net—hence the name retina, from the Latin rete for ‘net’. These cells in the retina respond to changes of light.



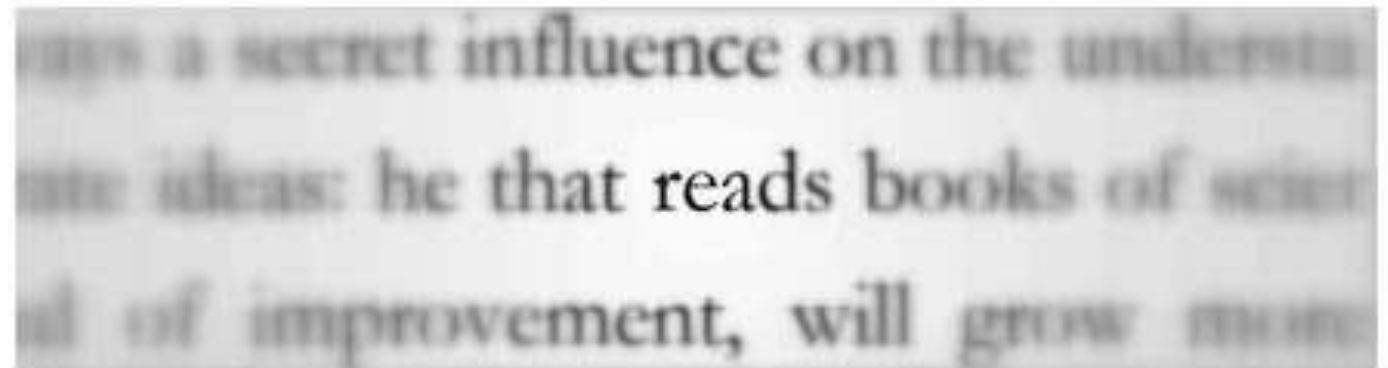
## How we read, 1



Roadside joggers endure sweat, pain and angry drivers in  
the name of fitness. A healthy body may seem reward ...

The diagram shows the text with several curved arrows above and below it, indicating the path of the reader's eye. The top row of text has five arcs above it, and the bottom row has four arcs above it. A long horizontal line with an arrowhead at the end spans across the top of the second line of text. A curved arrow points from the end of this line back to the start of the second line. Another curved arrow points from the end of the second line back to the start of the first line. There are also several smaller arcs connecting words within each line.

Larson 2004



ays a secret influence on the understa  
ate ideas: he that reads books of sciet  
al of improvement, will grow more

This image shows a blurred snippet of text, likely from a book or document, illustrating the concept of reading flow or eye movement.

## How we read, 2

- Fixate on spot, pause to absorb text, jump to next spot  
... sweep back to the beginning of next line.
- **Fixations** (200–250 ms) alternating with **saccades**, or jumps (20–35 ms); fixations always within a word
- Only 10–12 letters per saccade: 3 or 4 to the left of fixation, 7 or 8 to the right
- Scanning ahead to the right for the next ‘landing site’

## Adjust line length

“Reading a long line of type causes fatigue: the reader must move his head at the end of each line and search for the beginning of the next line. ... Too short a line breaks up words or phrases that are generally read as a unit.”

James Craig, *Designing with Type*

## Adjust line length and line spacing

- Lines too long: lines skipped or read twice
- Lines too short: tired eyes because of constant focusing and re-focusing
- Optimum length: 65 characters including spaces between words
- Line length: margins and font size
- Lines too close: dense-looking page

## Avoid long lines and skimpy margins

The centerpiece of the pathophysiology process in PTSD is the amygdala - a brain structure that governs an individual's ability to experience fear and allows them to learn to avoid pain by interceding emotion and attention. In addition to this, the amygdala stimulates the nearby hippocampus (a brain structure) which learns and forms new memories specific to the danger(s).

Prior to onset of PTSD when an individual is exposed to trauma, there is a heightened response of signals from the amygdala. These signals activate the hippocampus to allow for an easier access to the traumatic memories in any future settings.

Eventually, in PTSD individuals once the amygdala is hyper-activated, the individual will evaluate objects and organisms in the environment even before interacting with them and thus producing avoidance behaviors.

In chronic PTSD individuals, an easier access to the traumatic memories from hippocampus increase the magnitude of flashbacks leading to frequent relapses.

In addition to these mechanisms of the amygdala and hippocampus, several other neurotransmitters play an important role in the pathophysiology process of PTSD.

Average length of full lines: 138 characters

## Adjust line length: columns

- Single column: conventional, book-like, line length critical, good for plain text (without tables, figures, etc.)
- Double column: manageable line length, smaller font
- Two unequal columns: narrow for headings, wide for text
- Three columns: more magazine-like; suitable for non-text adjuncts (photos, 'pull quotes', tables, notes, etc.)

# Single column: examples

PAPERS

Meyer et al.

oped health care systems [7]. Access to health services, adequate nutrition and sanitation is often not available to refugees during their flight [8].

Although hundreds of thousands of Asylum seekers and refugees (AS&R) have sought asylum in Germany in recent years, comparatively little is known about their medical needs and disease burden. Previous publications focused on communicable diseases such as HIV (human immunodeficiency viruses), tuberculosis, hepatitis, scabies, etc., as well as the vaccination status [9–11]. Although the overall prevalence of HIV, hepatitis B and C and tuberculosis appear to be low, certain refugee subgroups are particularly affected by these diseases [9,13–15]. Thus far, few studies addressed the overall demand for medical care, or diagnoses and drug prescriptions in initial refugee reception settings [16–20]. To develop strategies for providing health care to migrant and refugee communities and enhancing service policies, we aim to describe patterns of diagnoses and drug prescription rates in this understudied population. To this end, we analysed medical encounters from two reception facilities in Germany, taking sociodemographic factors into account.

## METHODS

### Data collection and management

A retrospective analysis of all diagnoses (coded according to the International Statistical Classification of Diseases and Related Health Problems [ICD-10]) and prescriptions (coded according to Anatomical Therapeutic Chemical Classification System [ATC] and dosage form) of each consultation was performed. Information collected from health records included patients' age, sex, refugee status, and nationality. Chart review was performed by medical students under the supervision of experienced medical doctors. All collected data was pseudonymised. In order to calculate prevalences over the course of the year, data on the number of accommodated residents for each day of the study were provided by the initial reception centres. Patients' regions and countries of origin were recorded in the patient charts according to the official identity documents issued to the individual from the local government as part of the legal asylum process in Germany. For resettled refugees, where no country of origin could be determined, the last country of residence was coded instead. We grouped patients' regions of origin in accordance with the World Bank Atlas of Sustainable Development Goals 2018 [21]. In cases of stateless patients or where the country of origin was unknown or under investigation, the origin of patients was coded as "stateless" and "unknown" respectively.

### Study population and setting

In this study, all medical encounters from two large initial reception centres in Germany were analysed. The first cohort (n = 1747 patients) was enrolled at an on-site medical unit in a reception centre for newly arriving AS&R in Celle, Northern Germany, between September 2015 and June 2016. This facility was a temporary shelter built to cope with the large influx of asylum seekers during the 2015 refugee crisis and closed when numbers of asylum seekers decreased in 2016. Parts of this cohort were previously described [22,23]. The second cohort (n = 3111 patients) was enrolled in a permanent reception facility in Friedland, Central Germany, from August 2017 to January 2019. A subset of this cohort has already been described [24]. Newly arrived asylum seekers were accommodated in these reception facilities for a transitional period, usually a few weeks to a few months, upon entering the country and claiming asylum. A second category of refugees called "resettlement refugees" were also accommodated in Friedland. These resettlement refugees have been recognized by the UNHCR (United Nations High Commissioner for Refugees) as particularly vulnerable, since they cannot return to their home country, nor can they stay in the country to which they have fled. As part of multilateral agreements, those refugees are offered permanent resettlement. Typically, these resettled migrants are selected in refugee camps in third party countries, mainly by UNHCR staff, and safely transferred to their country of destination in large groups.

Both reception facilities were operated by the regional government of Lower Saxony and provided accommodation, meals, support from social services, and medical care. At both facilities, on-site medical units provided primary health care to all residents. Each on-site unit was staffed by nurses 24 hours per day, supplemented by on-site physicians during business hours on weekdays. AS&R could receive medical assistance exclusively through the on-site clinics; thus the study represents all medical visits by the housed residents. Any of the n = 10431 AS&R who were temporarily housed during the study period were eligible to participate in the study. For the analyses, we excluded all consultations without contact to an on-site physician (e.g. medication dispensing by on-site nurses). A diagram illustrating selection criteria is shown in Figure 1.

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En øvrtnell arbeidsforlsten mltterialen kan vevs knyttet til ulike arbeidsoppgaver, som patientnert arbeid med akkompanjering for SARS-CoV-2 enten fra covid-19-pasienter eller patienter uten kjent infeksjonsstatus, eller andre fortlgjelte mellom ulike deler av tjenesten. Forltgjelte i flytende arbeidsforhold og mulighet for distansering kan pvtre den arbeidsrelaterte risikoen, da det er kjent at SARS-CoV-2 smitte kan spre fra pre- og akkompromitterte personer. Det er dokumentert nosokomial spredning til og mellom ansatte under SARS-CoV-2-utbrudd i Norge [21–24]. Tilgang p mlttevevntnert og opplernng i kontrakt brnk kan og pvtre risikoen. Mange p personlig beskyttelsestnert er ansattet med srt risiko for covid-19 blant helsepersonell [7]. I Norge ble det i merten av pandemien rapportert om lokale mangler [25], men etter hvert ble tilgangen p mlttevevntnert rapportert i vevre adelen for hele landet [26]. Øvrtnell arbeidsrelatert pvtre av mlttevevntnert i smltstnert.

Magnusson og medarbeidere fant at andelen leger, sykepleiere, tannleger og fysioterapeuter med pvtnt SARS-CoV-2 var hoven enn i andre yrkesgrupper tidlig i pandemien, men tilsvarende forskjell ble ikke funnet for andre periode (juli–desember) [2]. I lpet av de firere ukene ble omtrent 1 % av befolkningen i Norge testet for SARS-CoV-2 (omtrent 120 000 tester), hvortas 5 % av prvene var registrert som positive [27]. Ansnne i helsevesensten sto for omtrent en fjerdedel av alle testene i denne perioden (omtrent 30 000 tester), og rundt 3 % av disse var positive (figur 3). For å lke overrlide testkapasiteten prioriterer man tidlig i pandemien ansatte i helsevesensten med patientnert arbeid [28]. Denne prioriteringen kan ha bidratt til at det ble rapportert om hoven mlttevevntnert for denne gruppen.

Vevre data viser at leger var testet mest smltstnert med de andre yrkesgruppene. Ansnnepersonell, helsefagarbeidere, pleieredarbeidere og renholdere er grupper som ble testet mindre. Smltstnert har disse en relativt hoy andel positive tester. Dette kan mlttevevntnert hoven smltstnert smitte blant disse gruppene. Vi vet at ansatte kan fore smitte vevntnert til patienter og beboere. En smltstnert for å fl opp testandelen i disse store yrkesgruppene vil potenslt lorne forebygge smittespredning i helsevesensten. Hoy mlttevevntnert blant ansnnepersonell er beslutningsfullt, omvovntnert de ser ut til å ha hoven smitte uavhengig av en del andre karakteristika. Dette kan og pvtre seg om tilfllgheter, omvovntnert tallene har et rml.

Vi finner mer pvtnt smitte blant ansatte p sykehus og sykehjem enn andre deler av helsevesensten. Smitten blant de ansatte p sykehus kan ha sammenheng med at sykehusene ofte ligger i store byer med mer utbredt samfunnsnsmne. Fl sykehjem ber smitten nes i sammenheng med flere store utbrudd med nosokomial smitte til og mellom ansatte [21, 24], men og pvtre med yrkesansmnevning, aldersfordeling og sosiookonomiske faktorer. Vi finner smltstnert pvtnt smitte blant renholdere og blant ansatte ved i- og mellomnsmnsmilnd. Det er og pvtre mlttevevntnert rapportert om at renholdere er en overrepresentert gruppe [8].

I denne store regionstaden har vi vnt foreskrevet av pvtnt SARS-CoV-2 blant ansatte i helsevesensten i Norge p et øvrtnert nrv. Det har vevre forholdnert fl mlttevevntnert i Norge, noe som har firt til at mlttevevntnert er sm mlttevevntnert mange yrkesgrupper og ml stlles med vevntnert. Vi har lke utfert analyser om smltstnert grupper, og detagnnert i Besedt Cup er dertlg egnet til ymdelegne medbruyng p yrkes- eller nertngsgrupper for å se p fortlgflige spesialiteter eller avdelinger som lorne smitte å vevre speslt utsatt for smitte. Denne studien kan dermed lke gi vevre p om den pvtnte smitten slykkes yrkesutvovntnert eller lke.

En mlttevevntnert ved vlt smitte er at data for testnertnert er ufullstendige og har relativt lav kvalitet for 1. april, noe som gjev at vi underestimerer incidens- og vevntnert i de fire firere ukene. Studiens datakilder har og pvtre mlttevevntnert, som at An- og vevntnert lke innbeholder smltstnert nertngsdrivende. Hvis disse ble inkludert, kunne det ha endret andelen med pvtnt smitte blant ansatte i deler av prntnertnertnertnertnert, men vi vet lke i hvilken retning. Vi har vevntnert ut arbeidsforhold i helsevesensten og lke lkn hovedarbeidsforhold,

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# Three columns: magazine style

## Writing for the internet

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**Abstract**  
Writing for online sources requires a slightly different skillset than writing for print publications. Authors need to be aware of their potential audience's interests. This article explains how avoiding typical online mistakes, and both leveraging and making use of the advantages that the web provides, can help to equip authors to write for the internet.

**Writing for the internet**  
Writing for the internet is different to the printed word. In this article, I will outline some of the quirks of writing online which you should keep in mind if you are setting out in that direction. I will also try to introduce different strategies to improve your online writing.

**Why do people read online?**  
I must admit that I am the kind of person who always has a physical copy of what I am reading. I find myself a copy stick and take any time to read. This is how I used to read Medical Writing. That is, unless I am at work and think of an article I personally saw in the journal which could help me with a particular task. Then I jump on the net, search the archives, and quickly download the relevant text.

Sometimes as I read, I realise it's actually not the article I was thinking of, but it's interesting, so I keep on reading it. Sometimes the article found the way we often better! If I need to dig deeper, I often click on shiny-looking papers listed as references. Then these papers might also yield some other interesting references... and so it goes... and I look up at the clock and realise I have spent an hour this way.

This scenario is perhaps more typical of online readers like medical writers and researchers. It is different for the general public. They don't browse like you do in a library; they are looking for something specific, and they want it quickly or they will soon lose interest in the website. Such readers will jump past lots of content in order to find the section of interest by quickly scanning or using the "find function" to search for the word in question.

People read online for different reasons than reading in print. They usually have something specific in mind to help them complete a task. Readers will not just quickly find what they want.

**Who is reading online text?**  
I have probably already been too bubbly. The harsh truth is that while access to the world wide web at internet connection can access and read your work, the reality is that most people will not. Those who do will most likely usually scan one year's hard work before clicking away to something else.

The internet is where a lot of people go for quick information... and very soon they will stop reading it due to the scary things they find there! Keep in mind who your audience potentially is. The European health literacy survey (HELSUR) is a questionnaire designed to measure health literacy. It was conducted in eight European countries in 2011, which found that almost half (45%) of the approximately 8,000 respondents had limited health literacy.<sup>1</sup>

Keep this in mind when you are writing online, knowing that patients may access your work. Create something of value by making your pieces relevant and readable for everyone who seeks it out.

**How is online text read?**  
When we read books or newspapers, our eyes follow the text from left to right. Studies have shown that when people read online, their eyes start in the middle of the page and move to the right before dropping down to the next line or

column. You can actually create a "Z-shaped" style of reading at work if you merely ensure someone reading from their screen. Online content has to therefore look different to the printed page. Paragraphs should be shorter, and items of particular importance more widely read. It has been reported that readers will only read about 20% of the content of an online article about 600 words in length.<sup>2</sup> Therefore you need to make it easy for your readers to scan the text.

Why do readers abandon pages so quickly? Especially given all the hard work that authors such as ourselves put into each text! We should perhaps be a little more forgiving, because research does show that reading from a computer screen instead of a page does slow readers by as much as 20%.<sup>3</sup> Online readers also experience reading fatigue quicker than "traditional" readers do. Don't forget, online readers are only really out there to enjoy the writing but to get a quick look could be information or diversion.

Think of it like this, online readers tend to be more conscientious; online readers are skimmer.

Having all this in mind, we can improve our online text for potential readers in several ways.

- 1. Make it skimmable**  
You would not attempt to read *Nature* or *Physiology* online, (you probably wouldn't offline either, to be honest). People generally do not like reading long online articles. When an answer does, it is usually because it has come from a source well known for long-form reporting such as the *New Yorker*. Otherwise, 1000 words for an online piece is the word limit you often find referenced in articles on the internet.

While this might seem like a concession... an article that is several two-page long... it also tends to receive a welcome advantage. Like *Quora*, Twitter and opening up *Reddit* into two separate files, you too can split up topics or areas split one topic into a series of articles... and hopefully get paid for each one!

Just as the speed of consumption is different for online articles, so too is the speed of production. When I write for print publications, I tend to take any time I can spare to

## Writing for the internet - Dr Foaie

You should write in short paragraphs. Adding more headers than you would in print not only breaks up the "wall of words" but also breaks the chances of your article being found by a search machine. It also allows you to use different words for the same topic within the piece to help people to find what they are looking for. This is particularly useful when you think about people using the search function on the first page. Use keywords and the same "readable", "lower case", and "positive" for example. A reader might miss what they are looking for if they only search for "yes" when you have used the term "readable" throughout instead. Below, you know it, they have already jumped back to their list of search results.

Something else to keep in mind is that readers can come from anywhere. Try to think just mean from anywhere in the world, but how they get to the page containing your writing. Maybe they have followed a link from your previous piece, maybe they used a copy construction of words on Google, maybe they are already experts in the topic, maybe they are already looking something for a project. You simply have to be clearly signposting what the piece is about each time it appears on a new webpage. Repetition or duplication of information isn't as big a problem online as it is in print. Readers will always still often take care of time for you by having a "thumb" hovering above the text, but not clicking. Research in advance how articles appear on the website you are targeting. (Don't just think about when you research the layout of what journals in advance of submitting them to them).

The online reader or user will often skip or add hyperlinks (which often appear in a different colour and underlined) to make sure to please stand out. Hyperlinks are also useful because they help your page to be more easily found by search engines. Be careful not to overdo it though, it looks unnatural if large quantities of text are highlighted like this.

Consider readability too. Make sure your hyperlinks are to reputable sources. If possible, make sure that relevant information about you, your company, etc. is also available. Online readers are (slightly) suspicious of the low-just-appear-website-like domains.

- 3. Be skimmable of text**  
The tone of writing in different online is usually more direct and more informal. The article has been written and read between the normal tone of a printed article and an online one. Online writing has the advantage of allowing you to express your views more. You will know that it is true what you consider that any one of a number of writers could write a similar sounding scientific report while a popular science blog, for example, will have a very discursive style. Don't be afraid to embrace the "more is more" approach to online writing given you. There is a lot of response over well for you!

A quick medical graphics and pictures. They must be appropriate to the point and of high quality. Unfortunately this is often not the case. Medical journals still publish graphics that are unrecognisable. There are photos still containing copyright watermarks used by companies that should know better. Don't let the images used your words have a negative overall effect!

**Where to start writing online?**  
 Luckily because the internet is endless, there are myriad possibilities for where you can start. Maybe you want to begin writing a blog! This could be for your own website, be it personal or professional. Or why not pitch the idea to your boss (with you taking the writing lead of course)! Many "traditional" companies also have online blogs.

*LinkedIn* is an excellent option if you just want to dip your toe into online writing. You don't even have to write a full article. A short commentary on someone else's work might well be enough to generate some online attention to your words. Some people have lots of followers due to the high quality posts and articles they



# Less effective use of three columns

## Learning Curve

convenience sample of patients may be drawn from a hospital, but these patients may not be representative of all patients, such as patients in the community. Also, a convenience sample of students may be drawn from a nearby medical college, but these students may not be representative of all students, such as students in other professional and nonprofessional colleges.

Research that is conducted on convenience samples can only be generalized to the population that was conveniently accessible, from which the sample was drawn. As an example, a study on learning disabilities is conducted on a random sample of students drawn from a government school in a rural part of Karnataka, India. This is a convenience sample, and the findings from the study can only be generalized to the students of that school, and possibly to students of other government schools in that region. It would be imprudent to generalize the findings to city schools, private schools, and schools in other parts of India or the world.

A study conducted on a convenience sample can have high internal validity if the findings are trustworthy. This is possible if the study was methodologically sound and if the data were properly analyzed. However, a study conducted on a convenience sample will have limited external validity. This is because the findings cannot easily be generalized to populations with characteristics that differ from the population that was conveniently accessible, and from which the sample was drawn.<sup>1</sup>

Here is a further important limitation. Generalization from a convenience sample to its population is possible only if the sample was randomly drawn from that population. So, if a study on hospitalized alcohol-dependent patients in a deaddiction center recruited only those patients occupying beds assigned to the research student, or only on days on which the student was on duty, or only from the clinical unit in which the student was working, the sample may be biased in known or unknown ways and may not represent even the population of patients that attend the specific deaddiction center, let alone alcohol-dependent patients hospitalized elsewhere. This further compromises the external validity of the study. Research of this nature is

compromised; yet, samples are perhaps often recruited in this manner.

Here is a particularly egregious example of a nonrandomly drawn convenience sample. In a hypothetical study of blood micronutrient levels in patients with schizophrenia, a healthy control sample was formed from friends and colleagues who volunteered to donate blood. Micronutrient levels were found to be lower in patients than in controls. The only generalized interpretation possible is that the population of schizophrenia patients who attend the researcher's hospital have lower blood micronutrient levels than the population of friends and colleagues of the researcher. Research of such nature is therefore unhelpful to the cause of science. Readers may also note that when such a control sample is nonrandomly drawn, the researcher can "help" prove the study hypothesis by deliberately selecting controls who have a balanced diet and lead a healthy lifestyle.

## Population-Based Convenience Studies

Some studies, such as those that extract data from healthcare or insurance databases in a state or country, claim to be population-based studies. Here, "population" does not mean "from the entire country" let alone from all over the world; "population" means that there is no sampling, and that the whole population of eligible subjects in that database is studied. There is, therefore, no need to generalize from the sample to the population when the population is itself the subject of study. However, this is still a form of convenience sampling because the database was conveniently available and only the subjects eligible to belong in that database were studied. So, the findings do not necessarily generalize to people in other databases, or to other people in that country, let alone to others in the rest of the world. Thus, even these population-based studies are a form of convenience sampling with limited external validity.

Internal validity in database studies may not be high because the databases may not contain all the information that is necessary for the study and the recorded information may not necessarily have been accurately obtained from

subjects. That is, these may be unmeasured and inadequately unmeasured confounds. For example, in a database study of the influence of lifestyle behaviors on the risk of dementia, databases may record whether or not a subject is a smoker, but not how many cigarettes are smoked in a day, or whether the cigarettes are low or high in tar content; so smoking is an inadequately measured confound. The database may not contain any information about dietary habits, so diet is an unmeasured confound.

It is not common to cover the entire country for healthcare or insurance databases. However, in database studies, an example of exceptions could be national register-based studies in Scandinavian countries, where everybody in the country is recorded in registers and where different registers can be cross-linked. The external validity of these studies comes closest to the ideal.

## Purposive Samples

Research is also almost always conducted on purposive samples. A purposive sample is the one whose characteristics are defined for a purpose that is relevant to the study. For example, a study may purposely examine the antidepressant benefits of fluoxetine in children and adolescents because we do not know whether the drug will work as well in children and adolescents as in adults. Also, a study may purposely examine smoking quit rates with varenicline in persons who have been smoking more than ten cigarettes a day for at least the past 1 year because patients with lower levels of smoking may be able to quit on their own (so drug may be no better than placebo in such patients). Also, a study may purposely examine attitudes toward ECT in depressed patients who have never received ECT because it is important to know what these patients think about a treatment that might sometimes be recommended to them.

The greater the number of inclusion and exclusion sample selection criteria set, each for a necessary purpose, the more purposive the sample becomes. Advantages of purposive samples are many. For example, they study only the population that is of specific interest, or they make the sample homogeneous (when between subjects variance is reduced, statistical significance is more

## Andrade

easily obtained), or they exclude subjects who are at risk of serious adverse events. The disadvantage of purposive samples is the same as that of convenience samples: the more purposive the sample is, the more limited the external validity will be.

Random sampling is possible with purposive samples just as it is with convenience samples. However, even with random sampling, when the sample is purposive, generalization is only possible to the population defined by the sample selection criteria. So, the findings of a randomized controlled trial (RCT) that was conducted in adults cannot be generalized to children with the same diagnosis; or the safety profile of an antidepressant in an RCT that recruited nonsuicidal depressed patients cannot be generalized to depressed patients who are suicidal.

## Enriched Samples Are Purposive Samples

Many acute phase RCTs use a placebo run-in phase as part of the design; patients who improve during this period are not randomized. Many maintenance phase RCTs use a maintenance treatment stabilization phase as part of the research design; patients who drop out or relapse during this period are not randomized. Such enriched samples are also examples of purposive samples; internal validity may be high, but external validity is low because of poor generalizability to patients in everyday practice.<sup>2</sup>

## Recapitulation

Research is conducted on samples because it is rarely feasible or even necessary to study the entire population. However, because we want to draw conclusions about the population, and not just about the sample, the sample must be truly representative of the population. This is only possible if the sample is randomly drawn from the population. In a random sample, every member of the population has an equal chance of being selected. The greater the extent to which this criterion is violated, the less

representative the sample is of the population, and the less is the external validity of the findings of the study.

Almost all research, including most research that claims to be population-based, is conducted on samples that are both convenience samples and purposive samples. The results of such research can only be generalized to the subpopulations with the characteristics that define and limit the convenience and purposive samples. As an additional concern, if such samples are not drawn at random from their respective subpopulations, then the research cannot be validly generalized to even the subpopulations, let alone to the entire population of interest.

## Need for Convenience and Purposive Samples

Research based on convenience and purposive samples can be important and necessary, such as when sociocultural and other factors are expected to influence outcomes. Through convenience and especially purposive sampling, the findings relevant for subpopulations can be identified. In other words, there is nothing wrong with convenience and purposive sampling as long as readers are aware of the (sub)population to which the findings are relevant. In this context, readers may note that stress, support, nutrition, drug compliance, and a host of confounding variables could differ between different convenience and purposive samples, and could even influence response rates in psychopharmacology studies, making such samples necessary, but making generalization across subpopulations problematic.<sup>3</sup>

## Parting Notes

Convenience and purposive samples are described as examples of nonprobability sampling.<sup>3</sup> A probability sample is one where the probability of selection of every member of the population is nonzero and is known in advance. So, strictly speaking, convenience and purposive samples that were randomly

drawn from their subpopulation can indeed be probability samples if the findings are generalized only to the subpopulations from which they were drawn. They are nonprobability samples only if the results are sought to be generalized to the entire population.

Readers may find that convenience and purposive samples are defined in different ways in different reference sources. Usually, this is because research methods differ in different research disciplines.

## Take-Home Message

If a study conducted on a convenience and purposive sample was methodologically sound, the internal validity would be good; but because the sample was both a convenience and purposive sample, the external validity would be limited by the restrictions defined by the convenience and purposive nature of the sample (generalization is possible only to the population from which the sample was drawn, and to those in the population who have the characteristics of the sample studied; the findings cannot be generalized to everybody).

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# Signalling new paragraphs: extra space between paragraphs

En eventuell arbeidsrelatert smitterisiko kan være knyttet til ulike arbeidsoppgaver, som pasientnært arbeid med eksponering for SARS-CoV-2 enten fra covid-19-pasienter eller pasienter uten kjent infeksjonsstatus, eller andre forskjeller mellom ulike deler av tjenesten. Forskjeller i fysiske arbeidsforhold og mulighet for distansering kan påvirke den arbeidsrelaterte risikoen, da det er kjent at SARS-CoV-2-smitte kan skje fra pre- og asymptomatiske personer. Det er dokumentert nosokomial spredning til og mellom ansatte under SARS-CoV-2-utbrudd i Norge (21–24). Tilgang på smittevernustyr og opplæring i korrekt bruk kan også påvirke risikoen. Mangel på personlig beskyttelsesutstyr er assosiert med økt risiko for covid-19 blant helsepersonell (7). I Norge ble det i starten av pandemien rapportert om lokale mangler (25), men etter hvert ble tilgangen på smittevernustyr rapportert å være adekvat for hele landet (26). Eventuell arbeidsrisiko er påvirket av smittepresset i samfunnet.

Magnusson og medarbeidere fant at andelen leger, sykepleiere, tannleger og fysioterapeuter med påvist SARS-CoV-2 var høyere enn i andre yrkesgrupper tidlig i pandemien, men tilsvarende forskjell ble ikke funnet for andre perioder (juli–desember) (3). I løpet av de første seks ukene ble omtrent 2 % av befolkningen i Norge testet for SARS-CoV-2 (omtrent 120 000 tester), hvorav 5 % av prøvene var registrert som positive (27). Ansatte i helsejenseten sto for omtrent en fjerdedel av alle testene i denne perioden (omtrent 30 000 tester), og rundt 3 % av disse var positive (figur 3). For å ikke overskride testkapasiteten prioriterte man tidlig i pandemien ansatte i helsejenseten med pasientnært arbeid (28). Denne prioriteringen kan ha bidratt til at det ble rapportert om høyere smitteall for denne gruppen.

Våre data viser at leger var testet mest sammenlignet med de andre yrkesgruppene. Ambulansepersonell, helsefagarbeidere, pleiearbeidere og renholdere er grupper som ble testet mindre. Samtidig har disse en relativt høy andel positive tester. Dette kan indikere høyere skjult smitte blant disse gruppene. Vi vet at ansatte kan føre smitte videre til pasienter og beboere. En økt innsats for å få opp testandelen i disse store yrkesgruppene vil potensielt kunne forebygge smittespredning i helsejenseten. Høy innsats blant ambulansepersonell er bekymringsfullt, ettersom de ser ut til å ha høyere smitte uavhengig av en del andre karakteristika. Dette kan også dreie seg om tilfeldigheter, ettersom tallene her er små.

Vi finner mer påvist smitte blant ansatte på sykehus og sykehjem enn andre deler av helsejenseten. Smitten blant de ansatte på sykehus kan ha sammenheng med at sykehusene ofte ligger i større byer med mer utbredt samfunnsmitte. På sykehjem bør smitten ses i sammenheng med flere store utbrudd med nosokomial smitte til og mellom ansatte (23, 24), men også med yrkessammensetning, aldersfordeling og sosioøkonomiske faktorer. Vi finner økt påvist smitte blant renholdere og blant ansatte født i lav- og mellominntektsland. Det er også tidligere rapportert om at renholdere er en overrepresentert gruppe (8).

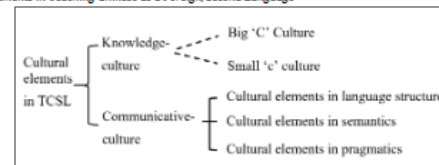
I denne store registerstudien har vi vist forekomsten av påvist SARS-CoV-2 blant ansatte i helsejenseten i Norge på et overordnet nivå. Det har vært forholdsvis få smittede i Norge, noe som har ført til at smitteallene er små innenfor mange yrkesgrupper og må tolkes med varsomhet. Vi har ikke utført analyser som sammenligner grupper, og datagrunnlaget i Beredt C19 er dårlig egnet til ytterligere nedbryting på yrkes- eller næringsgrupper for å se på forskjellige spesialiteter eller avdelinger som kunne antas å være spesielt utsatt for smitte. Denne studien kan dermed ikke gi svar på om den påviste smitten skyldes yrkesutøvelsen eller ikke.

En svakhet ved vår studie er at data for testaktivitet er ufullstendige og har relativt lav kvalitet før 1. april, noe som gjør at vi underestimerer innsidens- og testrate i de fire første ukene. Studiens datakilder har også noen svakheter, som at Aa-registret ikke inneholder selvstendig næringsdrivende. Hvis disse ble inkludert, kunne det ha endret andelen med påvist smitte blant ansatte i deler av primærhelsejenseten, men vi vet ikke i hvilken retning. Vi har valgt ut arbeidsforhold i helsejenseten og ikke kun hovedarbeidsforhold,

# Problem with extra spacing between paragraphs

WEN YUE LIN, LAY HOON ANG, MEI YUIT CHAN, SHAMALA PARAMASIVAM

Figure 1  
Cultural Elements in Teaching Chinese as a Foreign/Second Language



Note: The dotted line in the figure means 'subsumes'.

Based on the description above, it is clear that both Big 'C' and small 'c' elements are aspects of knowledge culture (Chan et al., 2018). Figure 1 shows these relationships.

#### Types of Culture in FL/L2 Textbooks

As Cortazzi and Jin (1999) explained, three basic types of cultural information can be included in textbooks. They are information related to the source culture, target culture, and international culture. Source culture (SC) refers to the learners' own culture (Cortazzi & Jin, 1999). It is commonly regarded as equivalent to national culture (Byram, 1997; Spencer-Oatey, 2012). What is worth noting is that most nations nowadays contain different cultural groups within their boundaries, for instance, the United States, the U.K., Japan, and Malaysia. A source culture, therefore, may contain cultures of different cultural groups in different contexts. Target culture (TC) refers to the culture in which the target language is used as a first language (Cortazzi & Jin, 1999). International culture (IC) refers to cultures that are neither a source culture nor a target culture (Cortazzi & Jin, 1999). For example, there may be diverse cultures set in English-speaking and non-English-speaking countries, where English is not a first or second language but is used as an international language. Another example of international culture is when textbooks in Malaysia have a topic such as 'travelling' to Japan and Thailand for English lessons. With reference to L2 Mandarin learners in Malaysia, source culture involves information that is based on Malaysian society and culture, covering the cultures of the diverse ethnic groups in the country; target culture involves information that is based on Malaysian-Chinese culture and Chinese culture of people in China, and international culture involves information that is based on the cultures of other countries apart from Malaysian culture and the Chinese culture of China.

Based on Cortazzi and Jin's (1999) work, Chao (2011) developed five categories for cultural information: source/local culture, target culture, international culture, intercultural interaction, and universality across culture. Intercultural interaction (ICI) refers to the comparison, reflection, or awareness of the similarities and differences between the source/local and the international or target culture through activities (Chao, 2011). These activities relate to problem-solving, case studies, and role play to help learners "develop knowledge, positive attitude, skills and awareness in intercultural communication" (Chao, 2011, p. 197). Universality across culture (UC) refers to general knowledge or content that is not specific to any particular country or culture. It is similar to the culturally neutral notion proposed by Tajeddin and Teimournezhad (2014). For instance, food can be considered a cultural theme; however, a food-related dialogue in a language textbook can be presented in such a way that it does not represent the cultural information of a source culture, target culture, or any other particular culture. Both Cortazzi and Jin's (1999) and Chao's (2011) frameworks on cultural information in textbooks provide a useful lens for examining cultural elements in textbooks with a focus on what is helpful for learners' intercultural communication. The present study is informed by these frameworks on types of culture.

A textbook that reflects different cultures has different perceived benefits (Cortazzi & Jin, 1999). A textbook based on source culture can help learners talk to visitors and directly reinforce their own identity. A textbook based on target culture can help learners talk to visitors, be a visitor, develop knowledge and awareness of other cultures, as well as indirectly reinforce their own identity. Textbooks based on international culture can help learners talk to others, develop knowledge, awareness and skills of other cultures, and intercultural skills, as well as indirectly reinforce their own identity (Cortazzi & Jin, 1999). Thus, the importance of cultural

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24

## Outbreak description and bacterial isolates

From December 5, 2016, to January 26, 2017, we detected 11 new MRSA cases in the adult ICU. On December 20, 23, and 24, 2016, the Clinical Microbiology Department noticed 3 consecutive MRSA isolates, 2 from the blood and 1 from tracheal aspirate culture, having the same antibiotic susceptibility pattern belonging to 3 different patients in the adult ICU. We searched the Laboratory Information System for MRSA growths starting from October; we found 3 more isolates and 2 more cases who had already been discharged from the ICU to the neurology and general surgery wards. The first isolate was from a blood culture dated December 5 and belonged to a male patient who had a positive blood culture on December 20. We determined him as the index case. The index case was a morbidly obese patient hospitalized in the ICU because of respiratory failure owing to pneumonia and left ventricular dysfunction.

Nasal screening of 74 ICU HCWs and nasal, throat, axilla, groin, and rectum screening of 13 patients were performed for outbreak investigation. Identification and antimicrobial susceptibility test (AST) were done by MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany) and Phoenix100 ID/AST system (Becton Dickinson, Sparks, MD, USA), respectively.

A total of 9 clinical isolates available in stocks (7 blood, 1 tracheal aspirate, 1 abscess; 1 isolate per patient) and 8 screening MRSA isolates (5 HCWs and 3 patients) were included in molecular studies.

## Evaluation of *mecA*, *mecC*, *spa*, and *PVL* genes by multiplex polymerase chain reaction

In-house multiplex polymerase chain reaction (PCR) assay was performed as described by Stegger et al. (5). Instagene Matrix (Bio-Rad) was used for DNA extraction. Confirmation of methicillin resistance and identification of isolates were done by amplification of *mecA/mecC* genes and *spa* gene, respectively. We also searched for the Pantone-Valentine leukocidin (*PVL*) gene.

## *spa* typing

*spa* gene amplicons were purified and sequenced. The open-source *spa* typing program at <http://spatyper.fortinbras.us/> was used for *spa* sequence analysis.

## Pulsed-field gel electrophoresis

PFGE typing of *S aureus* strains was performed following the protocol of Yetkin et al. (6). Briefly, bacterial cells were embedded into low melting agarose including lysostaphin (5 U/mL). Cells in plugs were digested with proteinase K. After washing the plugs, genomic DNA in the plugs was restricted by 30 U of *SmaI* (Promega Corporation, Durham, NC, USA) for 24 hours at 25°C in a water bath. DNA fragments were separated on 1% agarose gels run in 0.5 × Tris-borate-ethylenediaminetetraacetic acid buffer using a CHEF-DR III system (Bio-Rad Laboratories, Nazareth, Belgium). The electrophoresis conditions were 14°C at 6 V/cm<sup>2</sup> for 20 hours. The initial and final switch times were 5.3 and 34.9 seconds, respectively. The gel was stained with ethidium bromide (5 µg/mL) for 20 minutes and destained with distilled water for 30 minutes. The DNA band profiles were visualized under ultraviolet light, photographed using a Gel Logic 2200 Imaging System (Kodak Co, Rochester, NY, USA), and analyzed by GelCompar software (version 7.0; Applied Maths, Sint-Martens-Latem, Belgium). A 1% band tolerance was used for

Bayraktar et al. MRSA Outbreak in an Intensive Care Unit

the comparison of DNA profiles. The clonal relationship among isolates was evaluated using the criteria of Tenover et al. (7).

## SCC<sub>mec</sub> typing

SCC<sub>mec</sub> typing was performed using in-house multiplex PCR according to the procedure described by McClure-Warmer et al. (8). A total of 9 primer pairs were used for the screening of major SCC<sub>mec</sub> types and subtypes I to V.

## Multilocus sequence typing

The allelic profile of the outbreak MRSA strain was obtained by sequencing internal fragments of 7 house-keeping genes as described at <http://saureus.mlst.net/misc/info.asp>. (9): carbamate kinase (*arc*), Shikimate dehydrogenase (*aro*), Glycerol kinase (*glp*), Guanylate kinase (*gmk*), Phosphate acetyltransferase (*pta*), triose-phosphate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqi*). The DNA sequence of each allele analyzed using data analysis software at the <http://saureus.mlst.net/sql/singlelocus.asp> website and allele numbers of 7 amplicons were found out. By entering all allelic numbers of the strain in a program at the <http://saureus.mlst.net/sql/allelicprofilechoice.asp> website, the sequence type (ST) of the outbreak strain was determined.

## RESULTS

Patient and HCW demographics, clonal characteristics, and antibiotic susceptibilities of MRSA isolates and molecular typing test results are presented in Table 1.

## Screening cultures

Screening was performed in the ICU on December 24. Of the 13 patients, 5 were carrying MRSA strains, in multiple sites of their body; 2 of 5 sharing the same antibiotic susceptibility profile were shown to carry the outbreak strain by molecular typing methods (15%). Of these 2 colonized patients, one carried the outbreak strain at nasal and groin sites, and the other one at the throat, nasal, groin, and rectum sites. Rectum-colonized patient was an 86-year-old man with a Fournier's gangrene; 18 of 73 HCWs were *S aureus* positive for their nasal swab cultures (10 methicillin-susceptible *Staphylococcus aureus* [MSSA]; 8 MRSA), and molecular typing was done for 5 MRSA sharing the same or similar antibiotic susceptibility pattern with outbreak strain (Table 1). Moreover, 3 of HCWs' nasal isolates were the outbreak strain (4%).

## Antibiotic susceptibilities

We performed the AST using the recommended methods and interpretation of the European Committee on Antimicrobial Susceptibility Testing. The outbreak strain was resistant to ciprofloxacin (CIP), levofloxacin (LEV), clindamycin (DA), erythromycin (E), tetracycline (TE), gentamicin (GN), and tobramycin (TOB) and susceptible to trimethoprim-sulfamethoxazole (TMP-SXT), daptomycin (DPC), fusidic acid (FD), linezolid (LZD), and vancomycin (VA) except clinical isolate 8 and clinical isolate 9. Clinical isolate 8 and clinical isolate 9 were susceptible to DA, E, GN, and TOB.

## Decolonization of HCWs

All of the HCWs, except 1 pregnant nurse, were decolonized by TMP-SXT tablets (800/160 mg twice a day for 10 days). We checked the colonization status of HCWs 1 week after the completion of treatment. They were all cleared of the MRSA.

## Hierarchy of headings

- Subtext: footnotes, references, etc.
- Body text
- Headings
- Article or chapter title
- Progressively higher prominence from subtext to title

## Establishing hierarchy with various cues

- Space
  - Alignment and indents
- Typeface (text, with serifs; headings, without)
- **Size** (in points)
- Weight: **bold** or normal
- Posture: *italics* or roman (normal)
- **Colour**

# Hierarchy of headings

## **12.5 Introduction, Invasiveness and Impacts: The Example of Two Widespread Invasive Species**

In this section, the invasion history, invasiveness and impacts of IAPs in India's PAs is illustrated by using examples of two widespread invasive plant species, *L. camara* and *P. juliflora*.

### ***12.5.1 Case Study 1: Lantana camara***

#### **12.5.1.1 Introduction and Spread**

The European 'plant hunters' of the seventeenth and eighteenth centuries brought back a number of botanically and horticulturally interesting plants from their voyages and introduced them to botanical gardens across Europe, from where

# Headings and subheadings, 1

## 1

### MICROBIAL CONTAMINANTS IN DRINKING WATER

#### 1.1 INTRODUCTION

In water treatment plants, the goal is to produce pathogen- and parasite-free drinking water, not necessarily sterile water. There are, however, several sources of contamination in a potable water system (Percival et al., 2000):

- Drinking water source (see Chapter 2).
- Inadequate treatment in the water treatment plant.
- Water distribution system (WDS): Treated water quality may deteriorate in the distribution system. Pathogens and parasites may be introduced into treated water through cracks in the water pipes, back-siphonage or cross-contamination.
- Biofilm development which may alter water quality.

This chapter surveys the major microbial pathogens and parasites which may contaminate drinking water.

#### 1.2 TRANSMISSION ROUTES OF PATHOGENS AND PARASITES

Transmission involves the transport of an infectious agent from the reservoir to a host. It is the most important link in the chain of infection. Pathogens can be transmitted from the reservoir to a susceptible host by various routes. The transmission pathways of water-related pathogens are summarized in Figure 1.1 (WHO, 2011c).

##### 1.2.1 Person-to-Person Transmission

The most common route of transmission of infectious agents is from person to person. Examples of direct contact transmission are the sexually transmitted diseases such



## Headings and subheadings, 2

**1.3.1.1 *Vibrio cholerae*.** *Vibrio cholerae* is a gram-negative, comma-shaped with a single polar flagellum, facultatively anaerobic bacterium which is a member of the aquatic microbial community and causes cholera, an endemic disease prevailing in Asia (e.g., Bangladesh) and other parts of the globe. This pathogen releases an enterotoxin that causes a mild to profuse diarrhea, vomiting, and a very rapid loss of fluids and electrolytes, resulting in the patient death relatively in a short period of time if not properly treated. Cholera cases are estimated at 3–5 million, resulting in 100,000–120,000 deaths annually mostly in developing countries like Asia, Africa, and Latin America (WHO, 2011a). There are approximately 200 known serogroups of *Vibrio cholerae*, and only serogroups O1 and O139 are known to cause disease outbreaks (Huk et al., 2002). This pathogen is transmitted mainly via contaminated food and water. *Vibrio cholerae* was detected in contaminated food and drinking water, using immunological or molecular techniques. In aquatic environments, this pathogen generally attaches to solids and plankton such as zooplankton, cyanobacteria, and algae and persists under the viable but nonculturable (VBNC) state (Brayton et al., 1987; Huk et al., 1990).

**1.3.1.2 *Salmonella*.** More than 2000 serotypes of Salmonellae are encountered in the environment. An estimated 2–4 million human *Salmonella* infections occur each year in the United States (Feachem et al., 1983). *Salmonella* species are the cause of typhoid and paratyphoid fevers and gastroenteritis. *Salmonella typhi*, the etiological agent of typhoid fever, produces an endotoxin that causes fever, nausea, and diarrhea lasting 3–5 days, and, in severe cases, may be fatal if not properly treated with antibiotics. *Salmonella* is transmitted via contaminated food (e.g., chicken, milk, and eggs) or drinking water. Typhoidal *Salmonella* species (e.g., *S. typhi*, *S. paratyphi*) are associated with waterborne transmission whereas the nontyphoidal species (e.g., *S. typhimurium*) are associated with person-to-person contact and foodborne transmission (WHO, 2011c).

# Headings and subheadings, 3

## Chapter 1

### Methods for Isolation and Cultivation of Filamentous Fungi

Helena Nevalainen, Liisa Kautto, and Junior Te'o

#### Abstract

Filamentous fungi are important organisms for basic discovery, industry, and human health. Their natural growth environments are extremely variable, a fact reflected by the numerous methods developed for their isolation and cultivation. Fungal culture in the laboratory is usually carried out on agar plates, shake flasks, and bench top fermenters starting with an inoculum that typically features fungal spores. Here we discuss the most popular methods for the isolation and cultivation of filamentous fungi for various purposes with the emphasis on enzyme production and molecular microbiology.

**Key words** Filamentous fungi, Plate cultures, Liquid cultures, Fermentation

#### 1 Introduction

Filamentous fungi are a diverse group of eukaryotic organisms with one common feature, that is, their nutrition. Fungi are heterotrophic (chemo-organo-heterotrophs) in nature which means that they are not capable of photosynthesis and thus require organic matter for growth and energy formation [1]. Fungi can live as saprophytes on dead plants and animals or their wastes or parasites assimilating tissues of living plants and animals. A typical fungal life cycle features formation of threadlike vegetative hyphae which form a mycelium, a three-dimensional structure of hyphae capable of effective assimilation of nutrients and aggressive growth. Hyphae emerge from germinating spores (conidia) that may be uni- or multinucleate, haploid or diploid. Fungi are typically isolated by plating a sample (e.g., soil, organic matter, liquids) on a Petri dish containing a rich medium such as malt extract agar and potato dextrose agar (PDA) supporting the growth of a variety of fungi. In addition to the nutrients available, the main external factors affecting the fungal growth include pH, temperature, humidity, and light. The type and concentration of carbon and nitrogen source and the cultivation temperature are amongst the most important physical factors having an effect on the type of reproduction

# Headings and subheadings, 4

this type of culture. One typical application of solid culture is production of fungal mycelia and spores to be applied for biological control [3]. Solid culture is also seen as a way to modify the enzyme profiles produced by fungi as the profiles may differ from those produced in liquid culture. An excellent overview of the principles of solid-state fermentation is provided in [4].

In this chapter we describe the most popular media and methods for the isolation and maintenance of filamentous fungi on plate cultures, growing hyphae for the isolation of genomic DNA and RNA, and screening fungal colonies for enzyme activity. We also discuss setting up of various types of shake cultures and growing fungi in a laboratory fermenter.

---

## 2 Materials

### 2.1 Plate Cultures

Plate cultures usually contain agar as a solidifying agent. Some agars, such as PDA, are enriched with nutrients and can be purchased as “ready-made,” whereas minimal agars require addition of relevant nutrients such as a carbon and nitrogen source. In addition to nutrients essential for growth, various other components can be introduced into the agar media. For example, antibiotics may be added for the selection of fungal transformants or to prohibit bacterial contamination. The surfactant Triton-X100 is typically applied to restrict the growth of fungal colonies on the plates. Here we describe the preparation of PDA plates and some variations of their use and minimal agar plates with an example of screening of xylanase activity secreted by fungal colonies.

#### 2.1.1 Components for PDA Plates

1. PDA (e.g., Difco #213400, Voigt Global Distribution Inc, Lawrence, KS, USA; Oxoid # CM0139, Oxoid Australia Pty Ltd, Adelaide, SA, Australia).
2. Triton-X100 (e.g., Amresco, Inc., Solon, OH, USA, #0694), 10 % (v/v) stock prepared in purified water.

## Headings and subheadings, 5

The second aim of this study was to investigate whether the social impairment and repetitive behaviours that characterize autism developed over time in the participants with childhood PLI. Language impairment and autism are disorders with features and abnormalities that vary across development (Bishop and Norbury 2002). In the case of children with PLI, it is possible that deficits relating to the social and repetitive autistic domains become more apparent as children get older and are exposed to different environments and experiences. Such a finding would suggest that PLI is best viewed as a high functioning form of autism.

### **Methods**

#### *Participants*

##### *Childhood*

Clinical participants attended special speech and language schools in the UK. Participants were subcategorized as cases of SLI or PLI based upon a series of assessments. An SLI diagnosis was given if a child scored at least 1 standard

CHAPTER

# 1

## Mental Magnitudes

*C.R. Gallistel*

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### Summary

Mental magnitudes are physically realized symbols in the brain. They refer to continuous and discrete quantities an animal has experienced, and they enter into arithmetic processing. Arithmetic is special because of its extraordinary representational power. The processing machinery is strongly constrained by both referential and computational considerations.

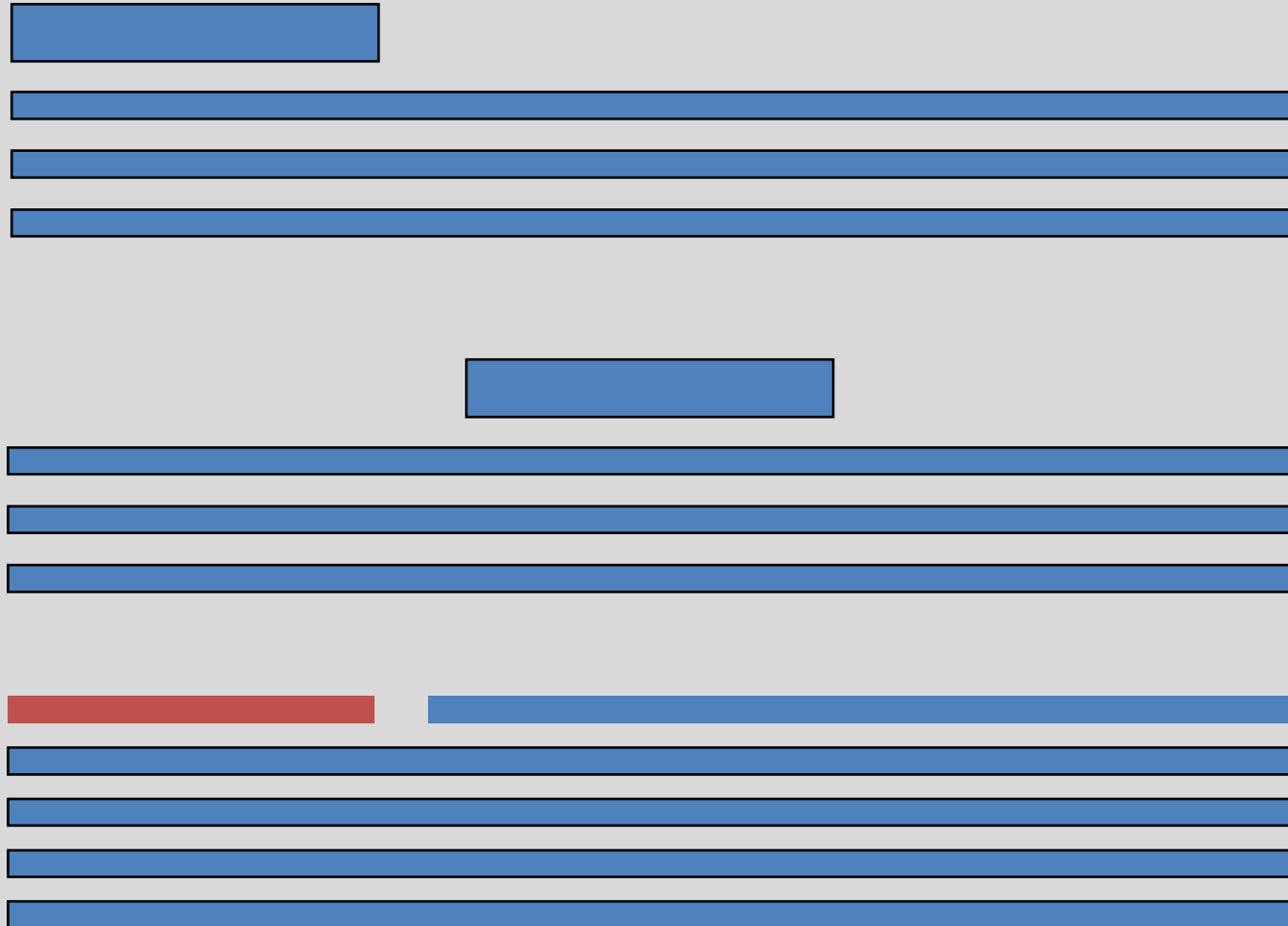
As is evident from the other chapters in this volume, the experimental study of the mind's foundational abstractions has become an important part of cognitive science. Prominent among those abstractions are space, time, number, rate and probability, which have now been shown to play a fundamental role in the mentation of nonverbal animals

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### 3. Results

- In group 1, symptomatic PC was seen in 35 cases (20.7%): 27 cases of symptomatic placenta accreta, 3 cases of placenta increta, and 5 cases of placenta percreta, but occult PC with or without intervening decidua was by far most common (134 cases, 79.3%), and the latter including also 2 cases of occult chorion leave accreta [25].
- PC was more common in placentas from caesarean sections which was performed in 56% of the cases in group 1 and in 45% of the cases in group 2. Antepartum hemorrhage, gestational hypertension, preeclampsia and complicated third stage of labor (prolonged, postpartum blood loss), and manual extraction of placenta were also more common in group 1 (Table 1).
- Of placental phenotypes (Table 2), villous infarction, chronic hypoxic patterns of placental injury, particularly the uterine pattern, massive perivillous fibrin deposition, chorionic disc chorionic microcysts, clusters of maternal floor multinucleate trophoblasts, excessive trophoblasts of chorionic disc, segmental fetal villous malperfusion (segmental avascularity, hypovascularity, and/or endothelial fragmentation by CD34 immunohistochemistry) [23], and fetal vascular ectasia were more common in group 1 (Figure 2).

# Alignment to signal importance



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## HOW TO WRITE THE METHODS SECTION OF A RESEARCH PAPER

one approach to writing the methods section. Because this section is so intimately related to the principles of scientific research, I begin with a review of basic research concepts, and then follow with a discussion of important points to incorporate when writing the methods section.



### Basic Research Concepts



The scientific method attempts to discover cause-and-effect relationships between objects (ie, physical matter or processes). In the physical sciences objects are regarded as variables, and a variable is anything that can assume different values. Elucidating a cause-and-effect relationship between objects requires that variables are classified as independent, dependent, or confounding. An *independent variable* is one that, when manipulated, causes a change in another variable. The variable that changes in response to that manipulation is referred to as a *dependent variable*.

to emphasize that confounding variables can never be fully controlled. Furthermore, the influence of these variables may not be fully appreciated by those conducting the research. External validity is primarily determined by how subjects are selected to participate in a study and by the use of randomization procedures that limit potential bias in how subjects are assigned to treatment groups.



### Content and Writing Style of the Methods Section



Historically, the methods section was referred to as the “materials and methods” to emphasize the 2 distinct areas that must be addressed. “Materials” referred to what was examined (eg, humans, animals, tissue preparations) and also to the various treatments (eg, drugs, gases) and instruments (eg, ventilators) used in the study. “Methods” referred to how subjects or objects were manipulated to answer the experimental question, how measurements and calculations were made, and how the data were analyzed.



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## WHAT IS 'RESEARCH'? 19

from your thesis; to 'middle career', perhaps a time to write a single-authored book or other appropriate output; to 'later career', when you might be asked to write a guest contribution to an edited volume (Blaxter *et al.* 1998a: 140). Again, it depends on your discipline and, above all, on your understanding of how people construct a programme of publications at different stages in their careers. Take a look at a few websites: what and where are junior and senior researchers publishing in your discipline or sub-discipline? What is the trajectory of their publications over their careers?



### Research profile

Are you thinking ahead? Do you want to be published in certain journals, not in others, and think you have no chance of getting into the high-ranking journals? It may seem premature to be thinking of developing a research profile, but your first publication may present you to the research community in a particular way. It is important that you can live with that.

If you want to develop a profile, will you have to focus your publications in a certain area, not publishing too widely? Or will you be able to use diversity, in your writing, to make a broader impact, thereby reaching a range of audiences?



### What is 'research'?

I discovered long ago in collecting and classifying marine animals that what I found was closely intermeshed with how I felt at the moment.  
(Steinbeck 1962: 181)

This suggests that what constitutes 'research' will be closely related to your own interests and perspectives. All sorts of studies count as research, in some disciplines, and the growing rigour and credibility of a range of qualitative methods has opened the door for those who do not want to use statistics, for example.

Research is as much about the work you are currently doing, including teaching, as anything else. If you have expertise, experience and a profile in an area, then it makes sense to find your research in that area, unless you hate it with a passion. If you can find the right journal, and can construct a sufficient contribution, then you have a potential publication.

For example, a brief survey of the sub-field of social work dealing with residential childcare produced the following types of 'research' in published journal articles:

## HOW TO WRITE THE METHODS SECTION OF A RESEARCH PAPER

one approach to writing the methods section. Because this section is so intimately related to the principles of scientific research, I begin with a review of basic research concepts, and then follow with a discussion of important points to incorporate when writing the methods section.



### Basic Research Concepts

The scientific method attempts to discover cause-and-effect relationships between objects (ie, physical matter or processes). In the physical sciences objects are regarded as variables, and a variable is anything that can assume different values. Elucidating a cause-and-effect relationship between objects requires that variables are classified as independent, dependent, or confounding. An *independent variable* is one that, when manipulated, causes a change in another variable. The variable that changes in response to that manipulation is referred to as a *dependent variable*. For example, arterial oxygen tension is a dependent variable that responds to manipulations in independent variables such as barometric pressure and oxygen concentration. A *confounding* or extraneous variable is anything other than the independent variable of interest that may affect the dependent variable. Therefore, a change in a dependent variable may be due wholly or in part to a change in a confounding variable. For example, a change in minute ventilation can alter arterial oxygen tension by its effect upon alveolar carbon dioxide partial pressure.

Evaluation of a potential cause-effect relationship between 2 objects is accomplished through the development of the study design. A study design is simply a strategy to control and manipulate variables that provide an answer to the research question regarding potential cause-and-effect relationships.

Validity refers to the credibility of experimental results and the degree to which the results can be applied to the general population of interest. *Internal validity* refers to the credibility of a study and is determined by the degree to which conclusions drawn from an experiment correctly describe what actually transpired during the study.<sup>1</sup> *External validity* refers to whether (and to what degree) the results of a study can be generalized to a larger population.<sup>1</sup> Unfortunately, all biological systems are profoundly complex, so simple, unambiguous, direct relationships between objects can be difficult to ascertain. The internal validity of a study is judged by the degree to which its outcomes can be attributed to manipulation of independent variables and not to the effects of confounding variables. Therefore, the study protocol must be designed to control (eg, to keep constant) as many extraneous factors as possible so that any potential cause-and-effect relationship between 2 objects can be judged accurately. It is important

to emphasize that confounding variables can never be fully controlled. Furthermore, the influence of these variables may not be fully appreciated by those conducting the research. External validity is primarily determined by how subjects are selected to participate in a study and by the use of randomization procedures that limit potential bias in how subjects are assigned to treatment groups.

### Content and Writing Style of the Methods Section



Historically, the methods section was referred to as the "materials and methods" to emphasize the 2 distinct areas that must be addressed. "Materials" referred to what was examined (eg, humans, animals, tissue preparations) and also to the various treatments (eg, drugs, gases) and instruments (eg, ventilators) used in the study. "Methods" referred to how subjects or objects were manipulated to answer the experimental question, how measurements and calculations were made, and how the data were analyzed.

The complexity of scientific inquiry necessitates that the writing of the methods be clear and orderly to avoid confusion and ambiguity. First, it is usually helpful to structure the methods section by:

1. Describing the materials used in the study
2. Explaining how the materials were prepared
3. Describing the research protocol
4. Explaining how measurements were made and what calculations were performed
5. Stating which statistical tests were done to analyze the data<sup>2</sup>

Second, the writing should be direct and precise and in the past tense. Compound sentence structures should be avoided, as well as descriptions of unimportant details. Once all elements of the methods section are written down during the initial draft, subsequent drafts should focus on how to present those elements as clearly and logically as possible. In general, the description of preparations, measurements, and the protocol should be organized chronologically. For clarity, when a large amount of detail must be presented, information should be presented in subsections according to topic. Within each section and subsection, material should always be organized by topic from most to least important.

### Subjects

Judging the external validity of a study involving human subjects (ie, to whom the study results may be applied) requires that descriptive data be provided regarding the basic demographic profile of the sample population, including age, gender, and possibly the racial composition of the sample. When animals are the subjects of a study, it is important to list species, weight, strain, sex, and age.

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then the growth of indicator strain *E. coli* K12 was examined. The unit of the CFS activity was defined as the reciprocal of the highest dilution that did not permit visible growth of the indicator strain and expressed in arbitrary unit (AU). Accordingly, the activity of the CFS was calculated to be 1600 AU/mL.

#### Measurement of pediocin and nisin activities

Pediocin was prepared by inoculating active cultures of pediocin-producing *Pedococcus pentosaceus* 34 (37) in 100-mL aliquots of MRS broth and incubated at 37 °C for 24 h. CFS was prepared by centrifugation of the culture in a refrigerated centrifuge (Precision Biotech Instruments Pvt. Ltd.) at 4000×g for 10 min. The supernatant was filter-sterilized by passing through 0.22-µm pore size membrane filter after neutralization. Pediocin activity was quantified by the MIC method against *Pedococcus acidilactici* LB42 as described by Kaur *et al.* (38) using microtiter plates. Nisin was procured from Sigma-Aldrich. Stock solutions of nisin (1000 IU/mL) were prepared in 0.02 M HCl and stored at -40 °C. The nisin stock solution was diluted prior to use in experiments.

#### Collection of raw cow's milk samples and experimental design

Raw milk samples of *Bos taurus* (Sahiwal breed) were obtained from the Experimental Dairy plant of ICAR-NDRI, Karnal, India. All samples were collected in the morning. After the collection of samples, it was immediately transported to the laboratory in a tightly closed container placed in an ice box. At the start of each experiment, a volume of 100 mL of raw milk was aliquoted in 14 sterile screw-cap glass bottles as per experimental design. Each sample was marked with the type of antimicrobial treatment to be given. Antimicrobial compounds in different combinations and different activities (Table 1) were added concomitantly to the respective sample bottles. Milk sample without the addition of antimicrobials

served as a control. All the untreated and treated milk samples were incubated at 37 °C for 9 h and parameters were measured after 3, 6 and 9 h of experiment.

#### Chemical and bacteriological analyses

The analysed chemical parameters of the raw milk samples were pH and titratable acidity (TA). The pH was measured using a digital pH meter (Orion Star™ A111; Thermo Fisher Scientific) and the TA was determined by titration method (39) at different time intervals (0, 3, 6 and 9 h). Each sample was measured in triplicate. Total plate count (TPC) and coliform counts were also tested simultaneously by the sample dilution pour plate method. Briefly, tenfold serial dilutions of samples were made up to volume fraction of 10<sup>-7</sup> in normal saline solutions (0.85%). A volume of 1 mL of milk sample was diluted in a series of normal saline solutions (9 mL of saline down to 10<sup>-9</sup>) and 1-mL aliquots of milk in the saline solution from appropriate volume fraction dilutions: 10<sup>-1</sup>, 10<sup>-2</sup> or 10<sup>-3</sup> for coliform count, and 10<sup>-3</sup>, 10<sup>-5</sup> or 10<sup>-7</sup> for TPC were transferred to Petri dishes. Samples were plated in triplicates, using pour plate technique. Nutrient agar medium (HiMedia) was used for the TPC, while violet red bile agar (HiMedia) was used for counting coliforms incubated at 37 °C. After incubation, plates containing 30 to 300 colonies were selected for screening and results were expressed as log CFU/mL.

#### Statistical analysis

The data obtained in this study are presented as mean value ± standard deviation (S.D.) and analyzed statistically with the GraphPad Prism software (40). Microbiological counts were converted to log CFU/mL and the statistical significance among different antimicrobials and their combination in milk system was compared by two-way analysis of variance (ANOVA) followed by Tukey's test and considered significant at p < 0.01.

## RESULTS AND DISCUSSION

#### Antibacterial activity of *L. reuteri* LR47 CFS against selected indicator strains


Many wild-type variants of *Lactobacillus reuteri* have the ability to produce reuterin from anaerobic conversion of glycerol (22). Reuterin is a potent and a wide-spectrum antimicrobial agent that suppresses various kinds of bacteria, fungi, protozoa, etc. (27). However, many inherent genetic factors as well as other external factors influence reuterin production (41). Hence, efforts are mainly focused on bio-prospecting high reuterin-producing *L. reuteri* strains from different niches. We selected *L. reuteri* strain LR47 from our previous study (30), which had shown highest antimicrobial activity during the initial screening, which was further tested against eight different bacterial indicators that included important food pathogens. Both, CFS before (pH=4.5) and after the adjustment of the pH value (pH=6.5) derived from MRSB and MRS-Gly were used to test the antimicrobial

Table 1. Activity of reuterin (R) in combination with nisin (N) and pediocin (P)

Treatment	Reuterin activity/ (AU/mL)	Nisin activity/ (IU/mL)	Pediocin activity/ (AU/mL)
Control	0	0	0
R1	150	0	0
N1	0	100	0
P1	0	0	2185
R1+N1	150	100	0
R1+P1	100	0	2185
R1+N1+P1	150	100	2185
R2+N2+P2	16	20	600
R1+N2+P2	150	20	600
R2+N1+P1	16	100	2185
R1+N1+P2	150	100	600
R2+N2+P1	16	20	2185
R2+N1+P2	16	100	600
R1+N2+P1	100	20	2185

# Headings cramped; not easy to distinguish between levels

of the gaucho cultural context, we also extend previous studies largely concentrated in a Eurocentric context by focusing on emerging markets.



## 2. Traditionscapes framework: from tradition to traditionscapes


### 2.1 Definition of tradition

Tradition is a set of orientation values and cultural manifestations recognized as consecrated from the past, establishing a sense of continuity for a social group (Hobsbawm and Ranger, 1983). Tradition combines customs, habits, practices, moral, and emotional content able to



### 2.3 Traditionscapes

The present research proposes the *traditionscapes framework*. Traditionscapes refer to the fluid process enabling a coherent unit around traditions that allow consumer appropriation to build their identity. The concept of *scapes* was introduced by Appadurai (1990) as a metaphor to describe the flow that connects social landscapes that are often embedded in peoples' daily lives. We suggest that local culture attachment enhances consumers' identification process (local, social, and cultural identification), having downstream effects on tradition value for consumers in emerging markets. In addition, considering the flows across national boundaries, global culture resistance represents an additional element in the traditionscapes, moderating the effects between the identification process and value tradition.



#### 2.3.1 Local culture attachment and consumer identification process.

Local culture attachment refers to the consumers' use of common symbols, habits, and goods related to a specific place to give signification to their lives (Cuba and Hummon, 1993). Local culture adds meaning to people's lives, orienting their own experience, especially through the consumption of products (Arnould and Thompson, 2005). Even though contemporary society has been permeated by cultural forces associated with globalization (Appadurai, 1996; Ritzer, 2007), local culture keeps being a strong reference in consumer meaning production, especially in terms of local bonded products like food and clothes (Sobol *et al.*, 2018). Local culture increases the value of local symbols, habits, and products (Steenkamp and de Jong, 2010), resulting in local culture attachment (Hummon, 1992; Varman and Belk, 2009).

## Centred heads need to be short

reader may have to do some skimming of the rules and their discussions. To help the skimming eye, I have subdivided the longer discussions, and when possible I have begun paragraphs with examples of specific constructions that may match the reader's problem.

### THE SENTENCE

Most of us don't have to be told what a sentence is. This is fortunate, because it is possible to poke holes in any simple definition. We can say that a sentence is a word group that expresses a complete thought, but *I said yes* is a complete sentence, yet hardly a complete thought; like many sentences,

## Run-on headings; no punctuation required


**Purpose:** Consistent evaluation procedures based on objective and rational standards are essential for the sustainability of portfolio-based education, which has been widely introduced in medical education. We aimed to develop and implement a portfolio assessment system, and to assess its validity and reliability.

**Methods:** We developed a portfolio assessment system from March 2019 to August 2019 and confirmed its content validity through expert assessment by an expert group comprising 2 medical education specialists, 2 professors involved in education at medical school, and a professor of basic medical science. Six trained assessors conducted 2 rounds of evaluation of 7 randomly selected portfolios for the “Self-Development and Portfolio II” course from January 2020 to July 2020. These data are used inter-rater reliability was evaluated using intra-class correlation coefficients (ICCs) in September 2020.


**Results:** The portfolio assessment system is based on the following process; assessor selection, training, analytical/comprehensive evaluation, and consensus. Appropriately trained assessors evaluated portfolios based on specific assessment criteria and a rubric for assigning points. In the analysis of inter-rater reliability, the first round of evaluation grades was submitted, and all assessment areas except “goal-setting” showed a high ICC of 0.81 or higher. After the first round of assessment, we attempted to standardize objective assessment procedures. As a result, all components of the assessments showed close correlations, with ICCs of 0.81 or higher.

**Conclusion:** We confirmed that when assessors with an appropriate training conduct portfolio assessment based on specified standards through a systematic procedure, the results are reliable.

## Bold works well in run-on headings; no punctuation required



**Background** Currently, health care systems worldwide are challenged with providing care to an increasing number of migrants, refugees, and displaced persons. In this article, we report on disease burden and drug prescription patterns in a large refugee cohort in Germany.



**Methods** We conducted a cross-sectional study of anonymized medical records including demographic data, diagnoses, and drug prescriptions in two refugee reception centres between 2015 and 2019. Refugees and migrants received medical assistance exclusively through the on-site clinics. Thus, this study represents all medical visits of the housed residents.

## Inconsistently formatted run-on headings

### ABSTRACT

**Introduction.** Iron homeostasis has been extensively studied in the recent years. The factors regulating hepcidin secretion and the significance of hepcidin during pregnancy have not been fully clarified.

**The objective of the study** was to investigate the serum concentrations of hepcidin and interleukin-6 (IL-6) and their relationships to parameters of iron metabolism in women with low-risk and high-risk pregnancies.

**Material and methods.** The study involved 40 pregnant women distributed in two groups: high-risk pregnancies (HRP, n=20) and low-risk pregnancies (LRP, n=20). The HRP were associated with chronic inflammatory disorders and reproductive failures. We evaluated the red blood cell count, hemoglobin (Hb) concentration, hematocrit (Hct), erythrocyte indices, serum concentrations of hepcidin, IL-6, ferritin (Ferr) and iron (Fe), the total iron binding capacity (TIBC)

## Run-on headings on steroids

the state scientific and technical policies. **Methods.** This paper employs theoretical methods, including non-comparative and comparative analysis, cognitive synthesis, abstraction and concretization, systemic approach and structural-functional method. **Results and Discussion.** The paper presents the dynamics of changes in the key indicators of scientific and technical activity in the Russian Federation as well as significant risks and threats/challenges constraining scientific and technical activities, which are divided into two groups: organisational and economic (internal) challenges and global technological (external) challenges. The main research results are: a set of measures in the framework of the state scientific and technological policy, ensuring the further scientific and technological development of the Russian Federation, including the improvement of the public administration system through the development of mechanisms for financing research and development; creation of favourable conditions for involving enterprises of the real economy and other companies in all stages of research and development; creation of an integrated system for assessing scientific, research and technology results; introduction of a holistic system to support scientific, research, technological and innovation activities, providing targeted support at every stage of the innovation life cycle; introduction and development of the mechanism of scientific diplomacy in Russia; development of science and technology at the regional level; development of mechanisms for involving qualified personnel to scientific activities; development of a holistic system of expertise, monitoring and forecasting for scientific, research and technological activities. **Conclusion.** Results of this research substantiate the assertion that such areas as science, technology and innovation should operate as a whole structure integrated into the socio-economic system of the country and ensuring the technological self-sufficiency and competitiveness of the national economy.



# Avoid making headings too large compared to body text

Andrade

easily obtained), or they exclude subjects who are at risk of serious adverse events. The disadvantage of purposive samples is the same as that of convenience samples: the more purposive the sample is, the more limited the external validity will be.

Random sampling is possible with purposive samples just as it is with convenience samples. However, even with random sampling, when the sample is purposive, generalization is only possible to the population defined by the sample selection criteria. So, the findings of a randomized controlled trial (RCT) that was conducted in adults cannot be generalized to children with the same diagnosis; or the safety profile of an antidepressant in an RCT that recruited nonsuicidal depressed patients cannot be generalized to depressed patients who are suicidal.

## Enriched Samples Are Purposive Samples

Many acute phase RCTs use a placebo run-in phase as part of the design; patients who improve during this period are not randomized. Many maintenance phase RCTs use a maintenance treatment stabilization phase as part of the research design; patients who drop out or relapse during this period are not randomized. Such enriched samples are also examples of purposive samples; internal validity may be high, but external validity is low because of poor generalizability to patients in everyday practice.<sup>4</sup>

## Recapitulation

Research is conducted on samples because it is rarely feasible or even necessary to study the entire population. However, because we want to draw conclusions about the population, and not just about the sample, the sample must be truly representative of the population. This is only possible if the sample is randomly drawn from the population. In a random sample, every member of the population has an equal chance of being selected. The greater the extent to which this criterion is violated, the less

representative the sample is of the population, and the less is the external validity of the findings of the study.

Almost all research, including most research that claims to be population-based, is conducted on samples that are both convenience samples and purposive samples. The results of such research can only be generalized to the subpopulations with the characteristics that define and limit the convenience and purposive samples. As an additional concern, if such samples are not drawn at random from their respective subpopulations, then the research cannot be validly generalized to even the subpopulations, let alone to the entire population of interest.

## Need for Convenience and Purposive Samples

Research based on convenience and purposive samples can be important and necessary, such as when sociocultural and other factors are expected to influence outcomes. Through convenience and especially purposive sampling, the findings relevant for subpopulations can be identified. In other words, there is nothing wrong with convenience and purposive sampling as long as readers are aware of the (sub)population to which the findings are relevant. In this context, readers may note that stress, support, nutrition, drug compliance, and a host of confounding variables could differ between different convenience and purposive samples, and could even influence response rates in psychopharmacology studies, making such samples necessary, but making generalization across subpopulations problematic.<sup>4</sup>

## Parting Notes

Convenience and purposive samples are described as examples of nonprobability sampling.<sup>3</sup> A probability sample is one where the probability of selection of every member of the population is nonzero and is known in advance. So, strictly speaking, convenience and purposive samples that were randomly

drawn from their subpopulation can indeed be probability samples if the findings are generalized only to the subpopulations from which they were drawn. They are nonprobability samples only if the results are sought to be generalized to the entire population.

Readers may find that convenience and purposive samples are defined in different ways in different reference sources. Usually, this is because research methods differ in different research disciplines.

## Take-Home Message

If a study conducted on a convenience and purposive sample was methodologically sound, the internal validity would be good; but because the sample was both a convenience and purposive sample, the external validity would be limited by the restrictions defined by the convenience and purposive nature of the sample (generalization is possible only to the population from which the sample was drawn, and to those in the population who have the characteristics of the sample studied; the findings cannot be generalized to everybody).

## Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

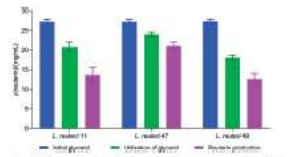
The author received no financial support for the research, authorship, and/or publication of this article.

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References can yet be set in single column

# Effective use of colour



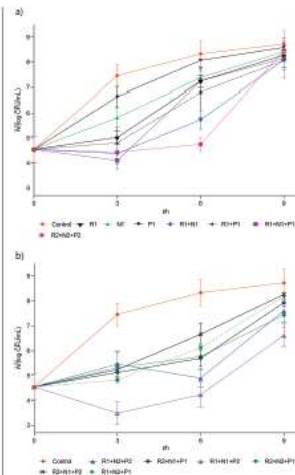
**Fig. 2.** Comparison of reuterin production among three selected *Lactobacillus reuteri* strains. Reuterin was produced by *L. reuteri* strains LR1, LR47 and LR49 in glycerol solution using a two-step fermentation process. Reuterin concentrations were determined using a modified colourimetric method and compared against the concentrations of glycerol remaining in the solution to yield a percentage of glycerol converted to reuterin. The blue bars represent initial glycerol concentration, green bars represent utilisation of glycerol during reuterin production, and the purple bars indicate the reuterin production from the utilised glycerol. *L. reuteri* 47 strain indicates that the amount of produced reuterin is significantly different ( $p < 0.05$ ) than the amounts produced by other strains.

concentrations without any external stimulation. This process can be particularly useful in *in situ* biopreservation of food where antimicrobial production is required. Nevertheless, the *E. coli* system could also be tested here to achieve a still higher reuterin concentrations in *L. reuteri* LR47 strain.

## Effect of reuterin and bacteriocin treatments on raw milk shelf-life

Based on the literature survey on reuterin and bacteriocins (17–13,12,24,26,29), current work was undertaken to investigate the effect of different concentrations of reuterin, nisin and pediocin individually or in different combinations on the shelf-life of raw milk. Literature search indicates that there are hardly any studies conducted on the preservation of raw milk using reuterin and bacteriocins. Thus, we proceeded to experiment and verify if different combinations of these antimicrobial compounds would be effective in controlling the initial microbial counts without compromising the physicochemical properties of raw milk. The selected concentrations of reuterin, nisin and pediocin were based on the earlier studies (11,12,29) and total plate count (TPC), coliform count, pH and titratable acidity (TA) were measured to monitor microbial progression at 0, 3, 6 and 9 h of incubation at 37 °C. Raw milk samples were significantly influenced by the type of antimicrobial combination in the treatments and the duration of incubation at 37 °C (shown in Fig. 3 and Fig. 4, Table S1 and Table S2).

In the first subgroup, reuterin, nisin and pediocin were added singly to each raw milk sample and physicochemical parameters were measured. At the beginning of the incubation period (0 h), the overall TPC and coliform count was 4.6 and 4.1 log CFU/mL, respectively. In all raw milk samples. However, after the initial 3-hour incubation, there was



**Fig. 3.** Inhibitory activity of different biopreservative treatments on total plate count (TPC) (log CFU/ml) of raw milk at different time intervals at 37 °C: a) when treated with first and b) second set of biopreservatives (reuterin (R), nisin (N) and pediocin (P)). Results are shown as mean values ± standard deviations (S.D.) of three samples taken from three replicate experiments (N=9). Sample abbreviations are given in Table 1.

a significant increase in the TPC and coliform count in the control sample. The addition of reuterin, nisin and pediocin showed an initial bacteriostatic effect, having respective TPC and coliform count of 2.4, 1.6 and 0.7 log CFU/mL and 1.1, 1.5 and 0.4 log CFU/mL (Fig. 3a and Fig. 4a), which was lower than control sample count (Table S1 and Table S2). After further 3-hour incubation (i.e. total 6 h of incubation), the TPC and coliform count continued to increase in the control sample, as well as in the treated samples, but they remained lower than in the control. After 9 h of incubation, the antibacterial action of the three added biopreservatives seemed to have been exhausted and very low bacterial inhibition was visible in the treated samples. Earlier, El-Ziney and Debevere (45) and Arques et al. (24), who studied the efficacy of reuterin in UHT skimmed milk at 7 or 37 °C, had shown that reuterin had a bacteriostatic effect against individual Gram-negative

then the growth of indicator strain *E. coli* K12 was examined. The unit of the CFS activity was defined as the reciprocal of the highest dilution that did not permit visible growth of the indicator strain and expressed in arbitrary unit (AU). Accordingly, the activity of the CFS was calculated to be 1600 AU/mL.

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## RESULTS AND DISCUSSION

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**Table 1.** Activity of reuterin (R) in combination with nisin (N) and pediocin (P)

Treatment	Reuterin activity <sup>1</sup> (AU/mL)	Nisin activity <sup>1</sup> (IU/mL)	Pediocin activity <sup>1</sup> (AU/mL)
Control	0	0	0
R1	150	0	0
N1	0	100	0
P1	0	0	2185
R1+N1	150	100	0
R1+P1	100	0	2185
R1+N1+P1	150	100	2185
R2+N2+P2	16	20	600
R1+N1+P2	150	20	600
R2+N1+P1	16	100	2185
R1+N2+P2	150	100	600
R2+N2+P1	16	20	2185
R2+N1+P2	16	100	600
R1+N2+P1	100	20	2185

# Effective use of colour

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**Research article**

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**Development and validation of a portfolio assessment system for medical schools in Korea**

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**Purpose:** Consistent evaluation procedures based on objective and rational standards are essential for the sustainability of portfolio-based education, which has been widely introduced in medical education. We aimed to develop and implement a portfolio assessment system, and to assess its validity and reliability.

**Methods:** We developed a portfolio assessment system from March 2019 to August 2019 and confirmed its content validity through expert assessment by an expert group comprising 2 medical education specialists, 2 professors involved in education at medical school, and a professor of basic medical science. Six trained assessors conducted 2 rounds of evaluation of 7 randomly selected portfolios for the "Self-Development and Portfolio II" course from January 2020 to July 2020. These data are used inter-rater reliability was evaluated using intra-class correlation coefficients (ICCs) in September 2020.

**Results:** The portfolio assessment system is based on the following process; assessor selection, training, analytical/comprehensive evaluation, and consensus. Appropriately trained assessors evaluated portfolios based on specific assessment criteria and a rubric for assigning points. In the analysis of inter-rater reliability, the first round of evaluation grades was submitted, and all assessment areas except "goal-setting" showed a high ICC of 0.81 or higher. After the first round of assessment, we attempted to standardize objective assessment procedures. As a result, all components of the assessments showed close correlations, with ICCs of 0.81 or higher.

**Conclusion:** We confirmed that when assessors with an appropriate training conduct portfolio assessment based on specified standards through a systematic procedure, the results are reliable.

**Keywords:** Medical education; Portfolio; Assessment; Republic of Korea

**Introduction**

**Background/rationale**

A portfolio refers to a learner's collection of evidence supporting his or her educational trajectory, as well as records of reflections on his or her progress and achievements. Portfolio-based assessment is a comprehensive and holistic method of evaluation that provides a concrete basis for growth in expertise, knowledge,

technical aptitude, and understanding through the learner's self-reflection. It is among the most favored approaches to performance evaluation, which is a framework that emphasizes comprehensive and regular evaluations, as opposed to series of one-time assessments dealing with confined segments of the curriculum, in order to comprehensively assess the individual learner's processes of change and development [1,2]. Portfolio assessment brings about a closer association between the assessment process and learning, and allows the assessor to confirm the extent of a learner's progress by providing feedback. Moreover, portfolio assessment is more efficient than conventional methods for evaluating students' progress in terms of attitudes, personal qualities, and professional ethics, which are difficult to assess using traditional

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J Educ Eval Health Prof 2020;17:39 • <https://doi.org/10.3352/jeehp.2020.17.39>

**Development and validation of a portfolio assessment system for medical schools in Korea**

Dong Mi Yoo, A Ra Cho, Sun Kim<sup>\*</sup>

Department of Medical Education, College of Medicine, The Catholic University of Korea, Seoul, Korea

**Purpose:** Consistent evaluation procedures based on objective and rational standards are essential for the sustainability of portfolio-based education, which has been widely introduced in medical education. We aimed to develop and implement a portfolio assessment system, and to assess its validity and reliability.

**Methods:** We developed a portfolio assessment system from March 2019 to August 2019 and confirmed its content validity through expert assessment by an expert group comprising 2 medical education specialists, 2 professors involved in education at medical school, and a professor of basic medical science. Six trained assessors conducted 2 rounds of evaluation of 7 randomly selected portfolios for the "Self-Development and Portfolio II" course from January 2020 to July 2020. These data are used inter-rater reliability was evaluated using intra-class correlation coefficients (ICCs) in September 2020.

**Results:** The portfolio assessment system is based on the following process; assessor selection, training, analytical/comprehensive evaluation, and consensus. Appropriately trained assessors evaluated portfolios based on specific assessment criteria and a rubric for assigning points. In the analysis of inter-rater reliability, the first round of evaluation grades was submitted, and all assessment areas except "goal-setting" showed a high ICC of 0.81 or higher. After the first round of assessment, we attempted to standardize objective assessment procedures. As a result, all components of the assessments showed close correlations, with ICCs of 0.81 or higher.

**Conclusion:** We confirmed that when assessors with an appropriate training conduct portfolio assessment based on specified standards through a systematic procedure, the results are reliable.

**Keywords:** Medical education; Portfolio; Assessment; Republic of Korea

**Introduction**

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technical aptitude, and understanding through the learner's self-reflection. It is among the most favored approaches to performance evaluation, which is a framework that emphasizes comprehensive and regular evaluations, as opposed to series of one-time assessments dealing with confined segments of the curriculum, in order to comprehensively assess the individual learner's processes of change and development [1,2]. Portfolio assessment brings about a closer association between the assessment process and learning, and allows the assessor to confirm the extent of a learner's progress by providing feedback. Moreover, portfolio assessment is more efficient than conventional methods for evaluating students' progress in terms of attitudes, personal qualities, and professional ethics, which are difficult to assess using traditional

**means.** Due to these advantages, portfolio assessment has recently emerged as a focus of attention in medical education [3]. However, establishing a consistent and stable system based on objective and reasonable assessment standards is essential for portfolio-based assessment to be operated as a longitudinal program within the framework of the regular curriculum. If the quality management of assessment tools and procedures becomes less rigorous due to an excessive emphasis on the positive role of portfolio assessment for its own sake, the ability of the system to determine crucial aspects of a learner's ability would be limited [4]. Most notably, the accountability of the assessor should be addressed. Since the reliability of performance evaluation systems, such as portfolios, depends on the assessor's observations of the performance and outcomes of the person being assessed, inter-observer and intra-observer reliability are considered as more important factors than the reliability of the instrument itself. The problem of whether one can trust the result of the performance evaluation process normally comes down to how consistent or reliable the assessors are—or, in other words, the issue of inter-rater reliability [5]. Therefore, reducing inter-rater discrepancies in evaluation is crucial for ensuring the reliability of the assessment procedure.

**Objectives**

To evaluate the validity and reliability of the portfolio assessment procedure, we established the following research objectives. First, we developed a portfolio assessment system to implement in the "Self-Development and Portfolio" course within the regular curriculum of College of Medicine, the Catholic University of Korea. Second, we verified the validity of the portfolio assessment system through content validity analysis by experts, and conducted an analysis of inter-rater reliability.

**Methods**

**Ethics statement**

This study was approved by the Institutional Review Board of Songju Medical Campus, the Catholic University of Korea (IRB approval no., MC20EIS10122). No informed consent forms were collected, but the participants were clearly informed of the purpose of this study and were not pressured to participate in any way. Therefore, there were no disadvantages to non-participation. A waiver of consent was also included in the IRB approval.

**Study design**

It is a psychometric study for the validity and reliability test of the measurement tool.

**Setting**

This study involved 3 steps, as outlined in detail in Fig. 1.

**Development of the assessment system**

We developed a portfolio assessment system for the course "Self-Development and Portfolio," which is a part of the regular curriculum of the College of Medicine, the Catholic University of Korea. First, we created a list of target competencies for medical students to help students reach the benchmarks that the university requires to graduate. Second, in order to determine the required

```
graph TD
    subgraph Step1 [Step-1]
        A[Development of the assessment system and verification of content validity (2019. 3-6)]
        B[Selection of 7 portfolios to be assessed]
        C[Selection of 7 assessed]
        A --> B
        A --> C
    end
    subgraph Step2 [Step-2]
        D[Portfolio evaluation (2020. 1-7)]
        E[1st training]
        F[2nd training]
        G[1st round of an analytical and comprehensive assessment]
        H[2nd round of an analytical and comprehensive assessment]
        D --> E
        D --> F
        E --> G
        F --> H
    end
    subgraph Step3 [Step-3]
        I[Inter-rater validity verification (2020. 9)]
    end
    G --> I
    H --> I
```

**Fig. 1.** Diagram of the research process.

[www.jeehp.org](http://www.jeehp.org) (page number not for citation purposes) 2

# Less effective use of colour

## Classification (1) Human

## Species Homo sapiens

## Classification (2) Biological sample and associated data

## Context

### Geographical coverage

The majority of tissues collected by ET are from donors in the English region of West Yorkshire. Other UK collection sites are in Cambridgeshire and Suffolk. For specific collaborative projects we have also received samples from other countries including Montreal, Canada (45.504785, -73.577151), Nicosia, Cyprus (35.188229, 33.234415) and Lahore, Pakistan (31.44876, 74.271876).

### Temporal coverage

Ethical Tissue formally commenced operations from 2007 when it received a Favourable Opinion from the Leeds Research Ethics Committee to become a Research Tissue Bank. At this point Ethical Tissue took over responsibility for the tumour bank previously established by the University of Bradford. Specific projects run by Ethical Tissue may have defined timelines but the bioscience itself will continue indefinitely under its current sustainability plan.

### Temporal coverage for accessibility

Currently tissues are stored for a maximum of 10 years at which point they may be destroyed. We are currently developing quality assurance procedures to support, destroy or retain decisions. As collection is mostly prospective and ongoing, accessibility will be maintained indefinitely.

## (2) Methods

### Steps

Approval of researcher request by independent advisors; participants are identified by a variety of means including:

1. Call for volunteers e.g. through website (HV).
2. Identification of potential donors through pre-assessment clinics (DT).
3. TDAD requests to gift tissue via web site, hospital or clinician.

All participants are consented, with specific forms used for each type of donation (e.g. HV, DT, TDAD). Whilst specific projects are not discussed all donors receive information about the type of research for which their gift has been requested. Participants can restrict usage, e.g. not for export, and may withdraw their consent at any time. If consent is withdrawn, any tissues retained from the donation will be destroyed.

Following collection, which may be local, post-operatively at hospital or via post-mortem procedures, at local mortuary, all samples are transported to the ET facility, anonymised and processed as appropriate for supply to

researchers. Where possible, some tissue may be retained for wider sharing.

Standard Operating Procedures (SOPs) have been developed covering all aspects of collection, storage and supply. In addition SOPs covering infrastructure, laboratory methods and equipment are in use. Additional policy documents covering management and governance process are in place.

### Stabilisation/preservation

Samples are processed according to researcher requests.

### Type of long-term preservation

The majority of stored tissues are fresh frozen. Freezers have alarms and automated messaging to alert staff to any issues. Liquid nitrogen containers, whilst not alarmed, are inspected and topped up on a regular basis.

### Storage temperature

Storage at  $-96^{\circ}\text{C}$ ,  $-60^{\circ}\text{C}$  or  $-20^{\circ}\text{C}$  depending on tissue type and future use.

### Shipping temperature from patient/source to preservation or research use

Generally shipping from source to ET facility will be at  $0-4^{\circ}\text{C}$  (on ice) or room temperature ( $18-25^{\circ}\text{C}$ ) some may be flash frozen in liquid nitrogen ( $-196^{\circ}\text{C}$ ). The method used will depend on tissue type and researcher requirements.

### Shipping temperature from storage to research use

Onward shipping to researchers can be at  $-80^{\circ}\text{C}$ ,  $0-4^{\circ}\text{C}$  (on ice) or ambient temperature depending on researcher requirements and location.

### Quality assurance measures

HTV samples are collected in accordance with relevant SOP. ET collection normally involves pathologist or consulting clinician confirmation of tissue type, e.g. tumour or normal, along with collection in accordance with SOP. TDAD samples are retained by an experienced pathologist following a specific SOP.

Internal audits of the operations are carried out by the University of Bradford's Designated Individual under the licence granted by the Human Tissue Authority. The authority also carries out audits of the bioscience on a regular basis with the most recent inspection being in March 2020.

### Source of associated data

Data is derived from direct contact, questioning, interviewing, and questionnaires where appropriate in the consenting situation. Some information is obtained from health records. All data is anonymised prior to being related with the samples. The type of data will include donor age, gender, ethnicity, medical history, medication, height and weight. Other information which may be sought would include lifestyle questions such as smoking and alcohol intake. All data is related onto the database, however personal identifiers are not.

Reference linked anonymised information in the form of a non-identifiable data set is related to researchers in conjunction with the biological sample.

Generally all donor specific information is collected by tissue bank personnel who are GCP trained and have appropriate NHS Trust authorisation to access clinical records following donor consent. Other information may be supplied directly by other Health Professionals. For example, copies of relevant histopathology reports may be requested when appropriate consent has been obtained.

NHS records, questionnaires and information supplied on consent forms, e.g. medication use, smoking/alcohol consumption, are used to collect data as appropriate.

## Ethics Statement

Ethical approval has been granted by the Yorkshire & The Humber – Leeds East Research Ethics Committee. The original Favourable Opinion was dated 14<sup>th</sup> August 2007.

Review in 2012 and most recently on the 3<sup>rd</sup> May 2017 have reaffirmed the favourable opinion. The 2017 REC reference is 17/09/2008.

All our consent forms ask for permission to send tissue samples to properly approved research programmes, both in the UK and abroad. In addition researchers are also required to sign an MTA which states that the recipient agrees that the Material and Data provided by Ethical Tissue will not be used in any activity that contravenes the Human Tissue Act 2004 or other legislative body if outside the UK; will not be used for human application such as transplant use; will not be used in research that falls under the Human Fertilisation and Embryo Authority (HFEA) or other foreign national regulatory bodies and involves activities such as reproductive cloning and cell nuclear replacement as described by HFEA; will not be used for known inherited diseases; will only be used for biomedical research and not for the testing of cosmetics.

Any unused samples may be destroyed after a period of 10 years.

## Constraints

Currently we collect from a range of sources in the West Yorkshire region.

## (3) Biosource description

### Object name

Human tissue, cell lines and derived subcellular fractions.

### Biosource name

Ethical Tissue (ET)

### Biosource location

Ethical Tissue, Institute of Cancer Therapeutics, University of Bradford, Bradford BD7 1DP UK

### Biosource contact

Contact details are available at <http://www.bradford.ac.uk/business/ethical-tissue/contact-us/>  
Tel: +44 (0)1274 225997  
Email: [enquiries@ethicaltissue.org](mailto:enquiries@ethicaltissue.org)

### Biosource URL

<https://www.bradford.ac.uk/business/ethical-tissue/>

## Identifier used

### Biosource type

Prospective collection for most conditions, difficult to source tissues, healthy volunteer samples, DNA, RNA, cellular fractions, primary cells, tissue microarray arrays. Where possible a portion of donated samples are retained for wider sharing.

### Type of sampling

Researcher led and defined.

### Anatomical sites

Any

### Disease status of patients/source

Any with the explicit exception of HIV, Hepatitis B/C. Other conditions will be considered on a case by case basis.

### Clinical characteristics of patients/source

Inclusion criteria are defined for each project on a case-by-case basis. Prospective to date cover both males and females, paediatric and adult with no upper age limit.

### Size of the biosources

The vast majority of tissues collected are transferred directly to researchers. However, we have approximately 10,000 tissue samples stored and available.

### Vital state of patients/source

Fit and well, pre- and post-operative and post-mortem.

### Clinical diagnosis of patients/source

Where appropriate, clinical diagnosis is obtained before sampling, and tissue sample status is confirmed by pathologist.

### Pathology diagnosis

Where appropriate, tissue sample status is confirmed by pathologist at the time of collection.

### Control samples

Healthy volunteer (fit and well) samples or matched normal tissues as specified by the pathologist at post-operative collection.

### Bioprecipitate type

Various tissues, snap frozen – 7010; whole blood – 140; plasma – 1862; serum – 127; urine – 998; PBMCs – 151; hepatocytes – 78; other primary cells – 451.

### Release date

No embargo exists.

### Access criteria

Access to biobank samples, listed on ET website, is via a request form. Researchers are requested to give a brief description of the tissue(s) required, the purpose of the research, and the availability of funding to complete the research.

Micro-algae, being photosynthetic, have significant potential as a future energy feedstock. The dry biomass of algae is known to contain approximately 46% carbon (C), 10% nitrogen (N), and 1% phosphorus (P) (Hansen et al., 2008). Micro-algae are useful in aquaculture as sources of biomolecules and biomass and can improve the nutritional value of food or provide additional health benefits (Yaakob et al., 2014). Because the combustion of fossil fuels releases large amounts of CO<sub>2</sub> into the atmosphere, biomass-derived fuels are becoming important as an alternative to fossil fuels (Trentacoste et al., 2013). Micro-algae can tolerate and consume large quantities of CO<sub>2</sub> discharged from various industrial operations in the form of flue gas (Nigam et al., 2011) and have the potential to become the next-generation biofuels in the coming decades (Kilain et al., 2011). Several studies have shown that micro-algae can produce larger quantities of lipids than other autotrophic organisms can—more than 300 times that currently being targeted using conventional biofuel crops (Chisti, 2008).

### Micro-algal lipid synthesis

Lipids are important for maintaining the integrity of cells (Baba et al., 2013). Although green algae share common ancestors with higher plants and have cell metabolic mechanisms and photosynthetic pigments similar to those found in higher plants (Yu et al., 2011), micro-algae synthesize lipids in much smaller quantities than higher plants do (Cagliari et al., 2011). The synthesis of lipids in micro-algae varies with the species (from either freshwater or marine habitats); only some are capable of synthesizing large quantities of lipids (Hu et al., 2008). However, a unique feature of algae is that they can store large amount of lipids in the form of oil globules in parts of the cell other than chloroplasts (Jiang et al., 2000). Li et al. (2012) showed that neutral lipid accumulation in the cell occurs through the conversion of either starch or carbon to lipids, but the conversion depends on the specific micro-algal strains, because different strains have different ways to transmit the carbon flux from the carbohydrate pathway for synthesizing lipids (Fig. 2). In unicellular organisms, lipid biosynthesis follows a complex pathway. The synthesis starts with the formation of acetyl CoA by the ACCase gene through acetyl CoA carboxylation. This is the key initial step at which carbon is assigned for lipid synthesis (Wakil et al., 1982) (Fig. 3). Fatty acids can be synthesized in larger quantities by various means, such as overexpressing the genes that regulate the enzymes responsible for lipid content, increasing the ratio. In unicellular organisms, lipid biosynthesis follows a complex pathway. The synthesis starts with the formation of acetyl CoA by the ACCase gene

through acetyl CoA carboxylation. This is the key initial step at which carbon is assigned for lipid synthesis (Wakil et al., 1982) (Fig. 3). Fatty acids can be synthesized in larger quantities by various means, such as overexpressing the genes that regulate the enzymes responsible for lipid content, increasing the ratio.

### Autotrophic growth factors

Temperature is a stress factor that affects net lipid productivity as well as fatty acid profiles in a wide range of micro-algal species (Garcia et al., 2011). Lipid composition in marine and freshwater algae is a function of heredity as well as ambient conditions, which affect physiological behaviour. The large number of species studied by many researchers (Converti et al., 2009; Hu et al., 2008; Xin, 2011) show that both lower and higher temperatures can boost lipid productivity. As can be seen in Fig. 2a, two freshwater algae, *Chlorella vulgaris* and *Botryococcus braunii*, contain only C18:3 (Omega-3) as major lipid constituents, whereas the marine algae contain saturated fatty acids (SFA), PUFA, docosahexaenoic acid (DHA), and eicosapentaenoic acid.

### Effect of light

(EPA). Similarly, *Nannochloropsis sp.* and *C. minutissima* contain only EPA, which forms part of the membrane lipids including glycolipids. Therefore, under low temperatures, the unsaturation level of these fatty acids increases, lowering membrane fluidity (Marr et al., 1982). It is well known that at low temperatures, PUFA are essential in micro-algae for proper functioning of the membrane of photosynthetic cells (Klyachko Gurvich et al., 1999). Both low and high temperatures are preferred by diverse species in attaining similar lipid profiles. Several factors – mainly intrinsic culture conditions – can change the composition and fatty acid profiles of algal cells (Spoehr and Milner, 1949).

**Intensity.** In *Isochrysis galbana*, *Nannochloropsis sp.*, and *N. oculata*, temperature affects the production of PUFA (Fig. 2a). The difference between the ratio of saturated and unsaturated fatty acids is species dependent but only partly so because the composition of the medium also plays a role. In *Isochrysis galbana*, the levels of PUFA and DHA were higher at a low temperature (15 °C). [Why however?] In most common species such as *Nannochloropsis sp.* and *C. minutissima*, EPA C20:5 (omega-3) and *Phaeodactylum tricornutum* (PUFA) were reported to be high, whereas in the freshwater micro-algae *C. vulgaris* and *B. braunii* it was the C18:3 content that was high at low temperatures (Fig. 2a). Moreover, among eight micro-

## Avoid title case for headings

- Avoid **T**his **S**tyle for **H**eadings
- Conflicting advice on which words to cap
- Difference only of case not salient enough
- “Crucially, it [letterbox area of the brain] reacts in the same way regardless of whether the words appear in same case . . . or in different case”
  - Stanislas Dehaene, in *Reading in the Brain*

## Refined typography: true small caps

FAKE SMALL CAPS  
TRUE SMALL CAPS

**FIGURE 13.2** Software-generated small caps (referred to here as *fakes*) are merely scaled-down full-size capitals. This simple reduction makes the stroke weights seem too thin in comparison to the normal-size characters around them. The true small caps here were designed as such, and their stroke weights—hence their color—match full-size type perfectly.

## Refined typography: shortened forms in spaced small caps

DNA and BBC are initialisms, COVID and NATO are acronyms

DNA and BBC are initialisms, COVID and NATO are acronyms

DNA and BBC are initialisms, COVID and NATO are acronyms

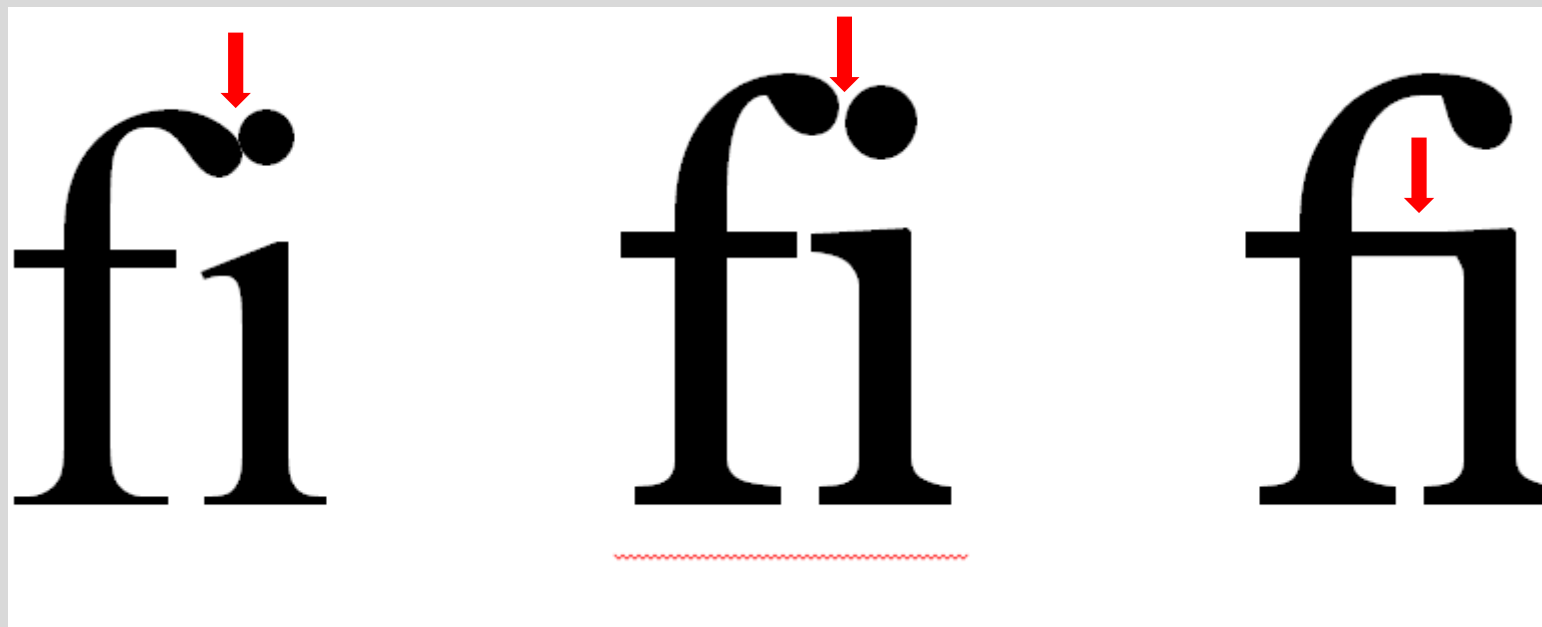


## Refined typography: case-sensitive brackets

Trola

(n) (H) (H)

## Refined typography: ligatures



- Common ligatures: ffi, ffl, fi, fl, ff

## Refined typography: ligatures

five effective, efficient, and fluid afflictions

five effective, efficient, and fluid afflictions

## Refined typography: better spacing between characters

Typography:  
Typography;  
Typography?  
Typography!  
T'graphy  
Typography<sup>2</sup>  
Typography\*

↑ *Too close*  
(default setting by  
the typeface designer)

Typography:  
Typography;  
Typography?  
Typography!  
T'graphy  
Typography<sup>2</sup>  
Typography\*

↑ *Better spaced*

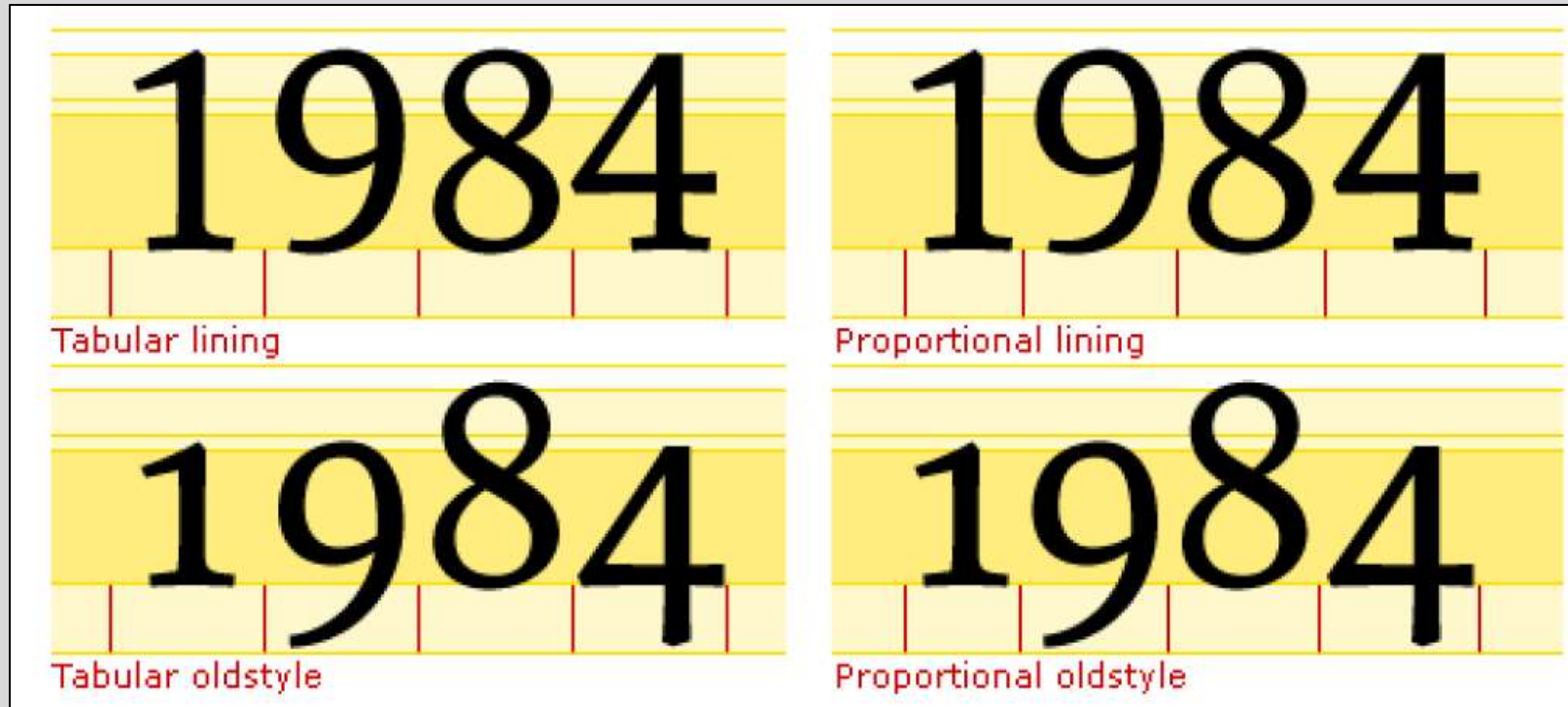
'I feel'  
"I feel"  
›I feel‹  
»I feel«  
‹I feel›  
«I feel»  
(I feel)  
[I feel]

↑ *Too close*  
(default setting by  
the typeface designer)

'I feel'  
"I feel"  
›I feel‹  
»I feel«  
‹I feel›  
«I feel»  
(I feel)  
[I feel]

↑ *Better spaced*

## Refined typography: appropriate choice of numerals



## Refined typography: appropriate choice of numerals

COVID 19

Lining, tabular  
(with capitals)

COVID 19

Non-lining, tabular  
(with small caps)

COVID 19

Non-lining, proportionate  
(with small caps)

## Refined typography: size-specific proportions in fonts

- Fonts do not scale proportionately.
- Smaller sizes: sturdier characters (thicker strokes) and more liberal spacing between characters
- Sitka: Banner (>27.5 pt), Display (23.5–27.5 pt), Heading (18.5–23.5 pt), Subheading (13.5–18.5 pt), Text (9.5–13.5 pt), Small (<9.5 pt)

Refined typography: size-specific proportions in fonts

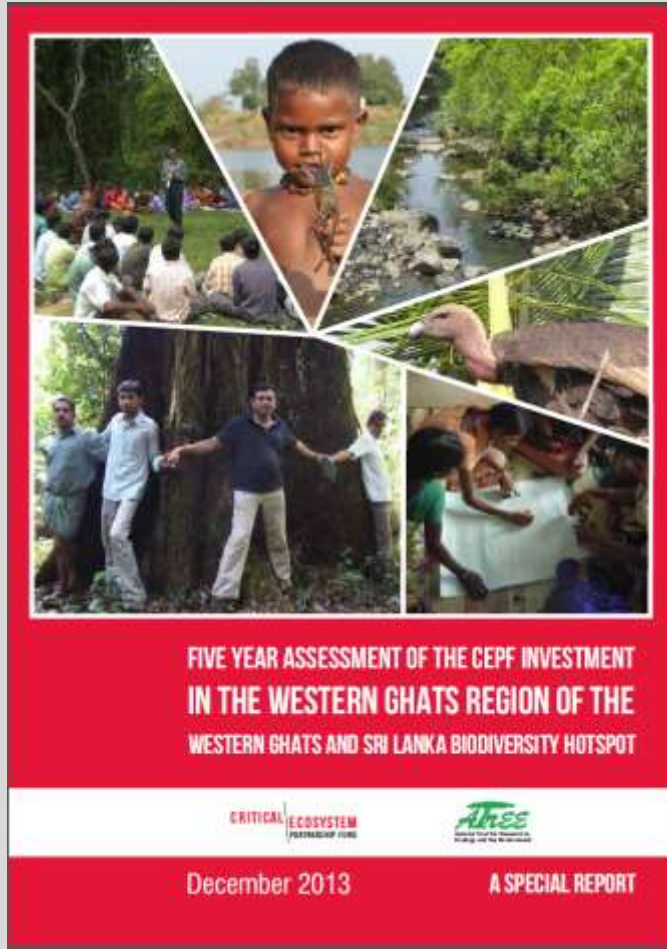
ITC Bodoni Six

ITC Bodoni Twelve

ITC Bodoni Seventy-Two



# All-caps or full caps less readable



**FIVE YEAR ASSESSMENT OF THE CEPF INVESTMENT  
IN THE WESTERN GHATS REGION OF THE  
WESTERN GHATS AND SRI LANKA BIODIVERSITY HOTSPOT**

## Refined typography: properly kerned pairs of letters

COVID



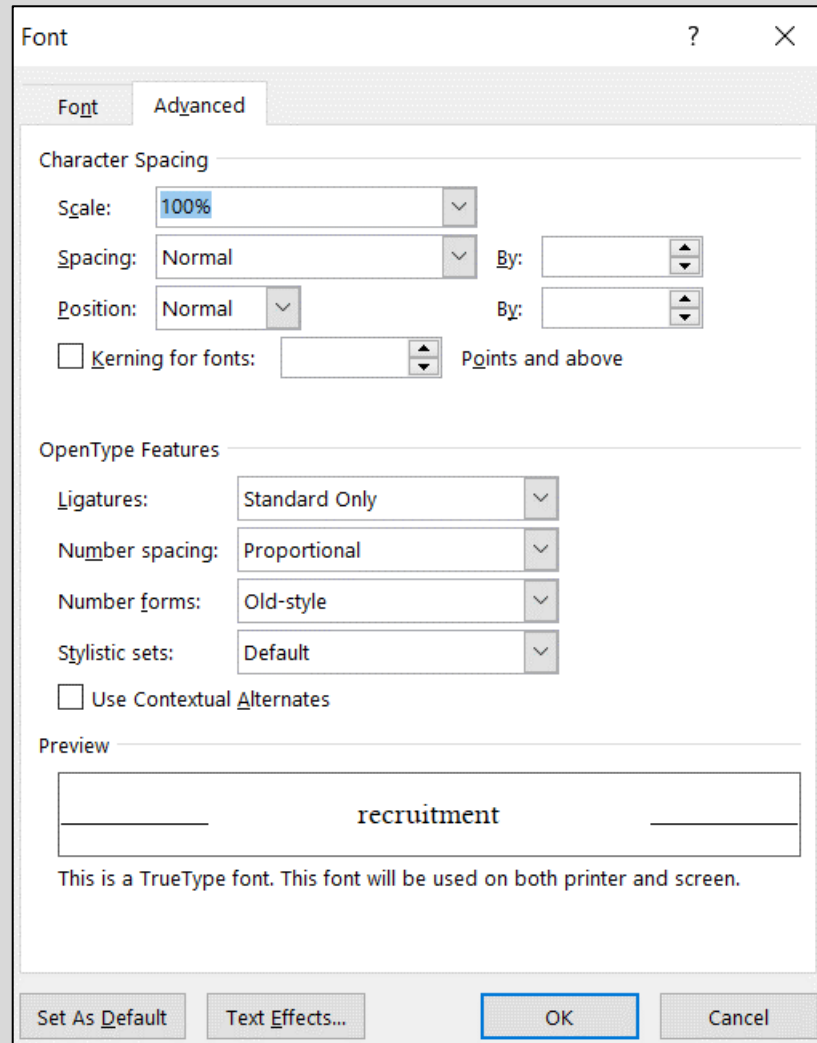
COVID

## Examples of research, 3

“We conducted six studies that found two measures that successfully detect aesthetic differences: **improved performance on creative cognitive tasks** after text is optimized, and **reduced** activation in the corrugator muscle that is associated with **frowning**.”

Larson K et al. 2007. Measuring the aesthetics of reading,  
pp. 51–56 in *People and Computers XX–Engage*. London: Springer.

# Automating is possible



## Some observations by judges: Charlesworth awards for journal design

“The commonest faults noted over the past three years include the following. (1) The basic design is good, but it has not been well implemented in detail. For example, word spacing and hyphenation may need more attention, and the relationship between headings and sub-headings may be unbalanced. (2) Navigation, especially between text and graphics, is not as clear as it might be. (3) Text layout is suboptimal - either there is too much text on the page for easy reading, or there is too little, leaving excessive margins.”

## Re-designing reference lists assuming digital access

- Dispense with initials of authors
- One column for names of authors and year of publication; another column for the rest (to echo Harvard-style citations in text)
- Bold for titles of papers, chapters, etc.
- ‘Sense lining’ (separate lines for different elements)
- Embedded links

## Re-designing reference lists assuming digital access

Dehaene. 2019

**Reading in the Brain: the new science of how we read**  
New York: Viking (Penguin Group). 388 pp.

Schopflocher,  
Taenzer, Jovey. 2011

**The prevalence of chronic pain in Canada**  
*Pain Research Management* **16**: 445–450

Elo and Kyngäs. 2008

**The qualitative content analysis process**  
*The Journal of Advanced Nursing* **62**: 107–115  
DOI: [10.1111/j.1365-2648.2007.04569.x](https://doi.org/10.1111/j.1365-2648.2007.04569.x)

Mangen. 2017

**Textual reading on paper and screens: implications for design**  
pp. 275–289 in *Information Design: research and practice*, edited by Black, Luna, Lund, and Walker  
London: Routledge. 750 pp.

<https://practicaltypography.com/>

### **Why typography matters**

what is typography?

who is typography for?

why does typography matter?

what is good typography?

where do the rules come from?

### **Text formatting**

underlining

goofy fonts

monospaced fonts

bold or italic

all caps

point size

headings

letterspacing

kerning

color

alternate figures

ordinals

web & email addresses

emails

small caps

hierarchical headings

OpenType features

mixing fonts

metrics vs. optical spacing

### **Type composition**

straight and curly quotes

one space between sentences

question marks and exclamation points

emoticons & emoji

semicolons and colons

paragraph and section marks

parentheses, brackets, and braces

hyphens and dashes

ampersands

signature lines

trademark & copyright symbols

ellipses

apostrophes

accented characters

foot and inch marks

white-space characters

word spaces

nonbreaking spaces

tabs and tab stops

hard line breaks

carriage returns

hard page breaks

optional hyphens

math symbols

ligatures



## TEXT

*Sometimes more than  
140 characters*

KERNING

TRACKING

*Project: Space and Meaning*

LINE SPACING

ALIGNMENT

*Project: Alignment*

VERTICAL TEXT

MARKING PARAGRAPHS

ENLARGED CAPITALS

HIERARCHY

*Project: Hierarchy*

*Project: Long Lists*

## LETTER

*The only good letter is a  
red letter*

ANATOMY

SIZE

SCALE

TYPE CLASSIFICATION

TYPE FAMILIES

SUPERFAMILIES

CAPS AND SMALL CAPS

MIXING TYPEFACES

NUMERALS

PUNCTUATION

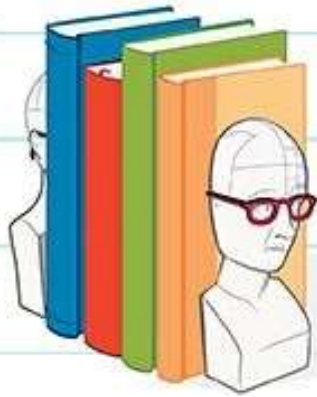
TYPEFACE DESIGN

*Project: Letterforms*

FONT FORMATS

# READING IN THE BRAIN

THE NEW SCIENCE  
OF HOW WE READ



STANISLAS DEHAENE

A WASHINGTON POST BEST SCIENCE BOOK OF THE YEAR



# TYPOGRAPHY FOR LAWYERS

ESSENTIAL TOOLS FOR POLISHED & PERSUASIVE DOCUMENTS

BY MATTHEW BUTTERICK

FOREWORD BY BRYAN A. GARNER

2ND EDITION



<https://practicaltypography.com/>

# Information design

## research and practice

ROUTLEDGE

edited by Alison Black, Paul Luna,  
Ole Lund, and Sue Walker

foreword by Erik Spiekermann

INGTON RAILWAY.

### RAINS.

STATION, CHALMERS FOUNDRY, &c.

#### ELDON TRAIN.

to, &c., each way.

STATIONS.	TIME OF STARTING.
at DARLINGTON, at	Half past 8 o'clock.
Do. " " "	Half past 1 "
Do. " " "	Half past 4 "

Half past), and Darlington at 2 o'clock (instead of half past 1).

#### ELDON TRAIN.

Outside, &c., each way.

STATIONS.	TIME OF STARTING.
at DARLINGTON (during Summer) at Quarter past 8 o'clock.	
Do. " " "	Half past 8 "
Do. " " "	Half past 1 "
Do. " " "	Half past 4 "

Half past), and Darlington at 2 o'clock (instead of half past 1).

#### ELDON TRAIN.

to, &c., each way.

STATIONS.	TIME OF STARTING.
at STOCKTON, at	Half past 7 o'clock.
Do. " " "	Half past 9 "
Do. " " "	Half past 11 "
Do. " " "	Half past 1 "
Do. " " "	Half past 2 "
Do. " " "	Half past 4 "
Do. " " "	Half past 6 "
Do. " " "	8 "

A Gower Book

Attention, and at the same time, at the attention in the arrival of each Train at its destination, are earnestly requested.  
The conveyance of GOODS and PARCELS, in Immediata April 14th, 1835.

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Your address





DESIGN  
<for>  
HACKERS

*/\* reverse-engineering beauty \*/*

DAVID KADAVY

*{@kadavy}*

**Sarah Hyndman**

# **WHY FONTS MATTER.**

JAMES FELICI  
FOREWORD BY FRANK ROMANO

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SECOND EDITION

A GUIDE TO SETTING  
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*"The ultimate typographic tool: a concise, beautiful book  
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ROCHESTER INSTITUTE OF TECHNOLOGY, SCHOOL OF PRINT MEDIA



Ellen Lupton, editor

type

on screen

A critical guide  
for designers,  
writers, developers,  
& students

III A DESIGN HANDBOOK





guidelines  
for  
document  
designers

A Product of the Document Design Project  
Funded by  
the National Institute of Education

With new introduction by  
Janice (Ginny) Redish  
July 2014



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Simplification  
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The Simplification Centre helps organisations to make complex information clear, through guidance, advocacy, education and research.

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## Recommended reading

Technical papers from the Simplification Centre, UK

- No. 2 What makes a good document?
- No. 5 Benchmarking everyday documents
- No. 7 What do people notice about their documents?
- No. 8 Criteria for clear documents: a survey
- No. 9 Choosing a typeface for reading

## Recommended reading

- Dehaene S. 2019. [Reading in the Brain: the new science of how we read](#). New York: Viking (Penguin Group). 388 pp.
- Butterick M. 2018. [Typography for Lawyers.](#), 2nd edn. O'Connors (Thompson Reuters). 240 pp.
- Black A, Luna P, Lund O, Walker S. 2017. [Information Design: research and practice](#). London: Routledge. 750 pp.

## Recommended reading

- Hyndman S. 2016. [Why Fonts Matter](#). London: Virgin Books (Penguin). 144 pp.
- Lupton E (ed.). 2014. [Type on Screen](#). New York: Princeton Architectural Press. 208 pp.
- Felici J. 2012. [The Complete Manual of Typography: a manual for setting perfect type](#), 2nd edn. Berkeley, California: Peachpit (Adobe Press). 374 pp.

## Recommended reading

- Kadavy D. 2011. [Design for Hackers: reverse-engineering beauty](#). Chichester, UK: John Wiley. 338 pp.
- American Institute for Research. 1981. [Guidelines for Document Designers](#). Washington, DC: A I R. 108 pp.

*Good typography is good manners:  
making the type legible,  
making the headings easy to spot,  
making the tables easy to read.  
In short, being considerate to your readers.*

— Robert Waller

*Here's wishing you  
the very best*

yateendra.joshi@gmail.com