

## 2.0 The Methods Section

Most science writers start with the methods section, sometimes called “materials and methods.” For most people, the methods section is also the easiest to write. Therefore, this book will present the methods section first. However, keep in mind that experimental or clinical procedures are not only presented in the methods section. The most important aspect of the methods may be mentioned briefly in the abstract and the introduction. In addition, key procedures often introduce paragraphs in the results section, i.e. by doing X procedure, we found Y result. Therefore, the contents of this chapter apply to descriptions of methods or procedures anywhere in a research report.

### 2.1 Structure of the Methods Section

The methods section has a simple pattern of organization. It tells the story of your research from beginning to end. Nevertheless, the methods section of clinical research reports requires a specific set of content, as detailed by the Information for Authors of the *Annals of Internal Medicine*:

For studies involving humans, describe in the Methods section how participants were assembled and selected, and the sites or setting from which they were recruited. Then describe study procedures including any interventions, measurements and data collection techniques. Use figures to diagram study processes including the flow of participants through the study. Provide the number of patients at each stage of recruitment and follow-up, including the number who declined to participate and the number who completed follow-up. State, if true, that an institutional review board approved the study or affirm that the protocol is consistent with the principles of the Declaration of Helsinki (World Medical Association), and state whether participants gave their informed consent. For studies that have numerical data and use statistical inference, include a section under Methods that describes the methods used for the statistical analysis and that states the specific statistical software. For all studies, include a statement at the end of the Methods section describing the role of the funding source for the study. If the study had no external funding source or if the funding source had no role in the study, state so explicitly.

Annals of Internal Medicine. Information for Authors.  
[http://www.annals.org/shared/author\\_info.html#manuscript-text](http://www.annals.org/shared/author_info.html#manuscript-text) . Retrieved 3 February 2008.

For basic science, the contents of the methods section may be quite different. The Information for Authors of the journal *Cell* focuses more on the reader’s ability to understand the author’s experimental procedures well enough to replicate them. This does not mean that all the details must be included in your own article. Common procedures should just be mentioned briefly with a citation to another article that describes them in detail.

The Experimental Procedures section needs to include sufficient detail so that readers can understand how the experiments were done, and so that all procedures can be repeated, in conjunction with cited references. This section should also include a description of any statistical methods employed in the study. A more detailed version of the procedures and details such as oligo sequences, strains, and specifics of how constructs were made can be included in Supplemental Data, but it is not appropriate to move the majority of the Experimental Procedures to Supplemental Data in order to shorten the text.

Cell Information for Authors. <http://www.cell.com/misc/page?page=authors#SubmissionRA> . Retrieved on 3 February 2008.

## 2.2 Expressions of Time

As you organize the story of your research project, some expressions of time will be necessary. Most writers use expressions of time (e.g., before, while, simultaneously) correctly until they focus on them. Then, they have a tendency to overuse them.

The most common expressions of time are adverbs: “also,” “then,” and “next.” Although it’s possible to use them at the beginning of a sentence, the more common location in research writing is next to the verb. Here are some examples.

### Examples:

OK: Then/Next we combined the two mixtures.

Better: We then/next combined the two mixtures.

OK: Also, blood clots were found in the legs.

Better: The blood clots were also found in the legs.

### Tip

When using adverbs of time, such as “also,” “then,” and “next,” in the middle of a sentence, the correct location depends on the verb. With forms of BE (am, is, are, was, were), the adverb follows the verb. With all other verbs, the adverb comes before the verb.

Using anesthesia is also recommended.

We also recommend using anesthesia.

In the first example below, expressions are usually placed at the beginning of sentences. Several short sentences have no connection to the sentences around them. Although there are no grammar errors, the style is not ideal.

### OK Example:

Cells “burn” glucose at low temperatures. First, glucose is oxidized to pyruvate in 10 steps. It releases small amounts of energy at each step. Next, pyruvate is oxidized in most eukaryotic cells to CO<sub>2</sub> and water. This takes 9 steps. Then the electron transport system traps energy in ATP bonds. At that time, the products of the biological oxidation reaction (CO<sub>2</sub> and water) are the same as those obtained by high temperature burning, but much of the energy is trapped in ATP bonds.

In the following revision, short sentences have been connected to the sentences around them to show their logical relationship. For example, the first three sentences have been combined using “when” and changing the verb “releases” to “releasing.” Although one adverb (“Next”) is left at the beginning of a sentence for variety, the others (“first” and “then”) have been moved next to the verbs, which sounds more advanced. Finally, the last expression, “at that time,” has been replaced with a non-time expression. The last sentence is not actually related by time. Instead, it is explaining more about the nature of the process.

### Better Example:

When cells “burn” glucose at low temperatures, the glucose is first oxidized to pyruvate in 10 steps, releasing small amounts of energy at each step. Next, pyruvate is oxidized in most eukaryotic cells to CO<sub>2</sub> and water, which takes 9 more steps. The electron transport system then traps energy in ATP bonds. In this process, the products of the biological oxidation reaction (CO<sub>2</sub> and water) are the same as those obtained by high temperature burning, but much of the energy is trapped in ATP bonds.

## 2.3 Active and Passive Voice Verbs

The most basic purpose of the methods section, and method descriptions elsewhere in the text, is to describe your actions. Therefore, we can expect that verbs will be important in the methods section. In English, there are two basic ways to describe actions—by using active and passive verbs. When the person doing the action is not as important as the action itself, you can communicate this with a passive verb. The following section will explore active and passive verbs in detail.

### 2.3.1 The form of the active and passive voice

We normally use the active voice. The active voice puts the person or thing doing the action in the subject position. In this example, the nurse is the actor.

**Example:**

The nurse administered the medication.

The passive voice changes the word order. The object (“medication”) becomes the subject.

**Examples:**

The medication was administered by the nurse.

OR

The medication was administered.

Mentioning the actor (“nurse”) becomes optional. This is often useful in research writing.

### Passive Verb Grammar

The form of the passive voice verb is: BE + past participle (or “PP”)

“BE” refers to the past, present or future form of “to be”: was, were, am, is, are, will be.

The past participle is usually Verb + “ed,” but there are many irregular forms.

For a list of irregular past participles, see: <http://www2.gsu.edu/~wwwesl/egw/verbs.htm> .

### 2.3.2 Typically Passive Verbs

Some verbs are usually passive. Here are some examples:

#### To be born

We use “I was born in . . .” so often in normal conversation that most people do not realize the verb is passive. In fact, although it’s possible to say “The mother bore the baby,” it’s not very common. This is a classic example of the main reason for using a passive—to omit the actor (the person or thing doing the action). Everyone knows that your mother bore you, so you do not need to mention it.

On the other hand, sometimes in biomedical writing, it is necessary to discuss the mother. The following example is the title of a clinical case report about a baby with certain health problems.

**Example:**

Concurrent multiple morphea and neonatal lupus erythematosus in an infant boy born to a mother with SLE

Ohtaki N, Miyamoto C, Orita M, Koya M, Matsuo M. Concurrent multiple morphea and neonatal lupus erythematosus in an infant boy born to a mother with SLE. Br J Dermatol. 1986 Jul;115(1):85-90.

**Tip:**

In research writing, you may find the expression “boy born **to** a mother.” This is different than most other passive verbs, which use “by.” In the example above, the word “to” replaces “by.” “Boy born” is a shortened form of the passive expression “boy that was born.”

### **To be hospitalized**

The words “hospitalized” and “admitted” are passive, except when the emphasis is on the patient voluntarily entering the hospital.

Examples:

Passive: The patient **was hospitalized** for complications following outpatient surgery.

Active: The **patient admitted himself** to a drug treatment program.

Note: “Committed” has the opposite meaning. The patient has no choice.

Example:

The patient **was committed** to a psychiatric facility.

“Discharged” is typically passive as well.

Example:

The patient **was discharged** from the hospital when treatment was complete.

### **To be deemed**

The word “deemed” means “officially decided to be . . .”

Example:

The infant **was deemed** to be ready for solid food.

### **To be staffed**

Although “staff” is usually a noun, referring to people, “to staff” can be a verb.

Example:

The hospital **was staffed** by emergency personnel during the storm.

### **To be said**

Of course, “say” is also common as an active verb (e.g., “she said something”), but it has a unique meaning in the passive. It means “most people say” and can replace a less formal expression from conversation: “they say.” Depending on the context, it may refer to “the public” or “scholars in our field.”

Example:

It **is said** that acupuncture is more effective than medication for pain relief.

## **2.3.3 Verbs That Are Never Passive**

You’ve probably studied these rules before. However, when you are writing most of your paper in the passive voice, it is easy to accidentally make one of these errors. Use the active voice in all of these situations.

### **Containing Verbs**

(hold, comprise, lack, etc.)

Example:

Active:       ○ The flask **contains** a hazardous chemical.

Passive: X A hazardous chemical **is contained** by the flask.

The verb “consists of” seems to cause a lot of trouble for English learners. It is never passive.

**Example:**

Active: O The human heart **consists of** four chambers.

Passive: X The human heart **is consisted of** four chambers.

### Reflexive Action

**Example:**

Active: O The diabetes patient **administered** her own medication.

Passive: X Her own medication **was administered** by the diabetes patient.

### Reciprocal Action

**Example:**

Active: O Family members **supported** each other through the grieving period.

Passive: X Each other **were supported** through the grieving period.

### Intransitive Verbs

(fall, live, die, become, etc.)

This is the most common error in this category. Verbs without an object are called intransitive. They cannot be passive because there is nothing to use for the subject.

**Example:**

Active: O The temperature **fell**.

Passive: X The temperature **was fallen**.

**Tip:**

If you are trying to decide whether a verb can be changed to the passive, ask yourself this question: Is an object after the verb possible?

Example: ??? “The mouse ran.” OR “The mouse was run.” ???

Think: “The mouse ran *something*. No, that’s not possible. It doesn’t make sense to ‘run something.’ Therefore, the verb ‘run’ has no object, and it cannot become passive.”

Write: “The mouse ran.”

(Actually, “run” does exist with an object in English, but it has another meaning—to run a machine, for example.)

### 2.3.4 Verbs That May Be Active or Passive

Basically, the passive voice allows you to focus on the action or object of action. It also allows you to avoid mentioning the person or thing doing the action ( the “actor”), or at least take the emphasis off the actor. In descriptions of procedure, or methods, in particular, the action or object of the action is more important than the actor. Anyone in the lab can run an assay. Hopefully, all of them will get the same results. Therefore, not mentioning the lab worker makes the procedure appear more objective and puts the focus on the procedure.

**Examples:**

X Doxazosin **was titrated** at two-week intervals **by the lab assistant**.

O Doxazosin **was titrated** at two-week intervals.

On the other hand, if you need to mention the actor, you can use “by +actor.” In the following clinical study, the action is the main focus, but the qualifications of the clinicians are important, so they are mentioned in a “by-“ phrase.

**Example:**

Ultrasound-guided biopsies **were obtained by a radiology resident** (HD) working under the direct supervision of a board-certified radiologist (LN). . .

. . . All specimens **were examined by a board-certified pathologist** (EWH). . .

However, additional actions by the board-certified pathologist are listed in passive sentences.

**Example:**

. . . Glomeruli in each section **were counted**. A quality score **was** then **assigned** on the basis of whether the specimen was fragmented, appeared crushed, or was < 6 mm long. The presence of muscle throughout the specimen or constituting a part of the specimen **was noted**.

Diagnostic quality of percutaneous kidney biopsy specimens obtained with laparoscopy versus ultrasound guidance in dogs. Rawlings CA, Diamond H, Howerth EW, Neuwirth L, Canalis C. J Am Vet Med Assoc. 2003 Aug 1;223(3):317-21.

**Tip**

Sometimes it is not easy to choose between active and passive. If the researcher did the work, use a passive verb. If the object of research (patient, molecule, etc.) did something to itself, use an active verb.

In the example below, “was increased” is used to describe an experimental variable that the researcher controls i.e. deliberately changes. In this example, the researchers changed the sample size.

**Example:**

To protect against an underpowered comparison owing to either loss to follow-up or overestimation of the efficacy of high-intensity therapy, the originally planned sample size **was increased to** a total of 90 patients.

Crowther MA, Ginsberg JS, Julian J, Denburg J, Hirsh J, Douketis J, et al. A comparison of two intensities of warfarin for the prevention of recurrent thrombosis in patients with the antiphospholipid antibody syndrome. N Engl J Med. 2003 Sep 18;349(12):1133-8.

However, when simply indicating results the active form is used. This describes an outcome that the researcher did not deliberately manipulate.

**Example:**

Among men whose consumption **remained stable or increased**, a 12.5-g increase in daily alcohol consumption (as a linear variable) was associated with a relative risk of myocardial infarction of 0.78 (95 percent confidence interval, 0.62 to 0.99).

Mukamal KJ, Conigrave KM, Mittleman MA, Camargo CA Jr, Stampfer MJ, Willett WC, et al. Roles of Drinking Pattern and Type of Alcohol Consumed in Coronary Heart Disease in Men. N Engl J Med. 2003 Jan 9;348(2):109-18.

Thus “increased” and “was increased” can have totally different meanings depending on the sentence.

**Choose the correct form for each sentence below. Answers follow.**

1. Patients were randomly assigned to receive eplerenone (25 mg per day) or matching placebo for four weeks, after which the dose of eplerenone **increased/was increased** to a maximum of 50 mg per day.
2. After week 1, the mean systolic and diastolic blood pressure **increased/was increased** in both groups from base line to each time point throughout the remainder of the trial.

Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, et al. Eplerenone, a Selective Aldosterone Blocker, in Patients with Left Ventricular Dysfunction after Myocardial Infarction. N Engl J Med. 2003 Apr 3;348(14):1309-21.

**Answers**

1. The dose of eplerenone was increased. The researcher did it.

2. The blood pressure increased. It happened naturally.

Note: Objects of research (patients, molecules, etc.) may also appear in a “by-“ phrase, if they act on another object. In the journal article title below, the acetylcholine work to relax the muscle.

**Example:**

The obligatory role of endothelial cells in the relaxation of arterial smooth muscle **by acetylcholine**

Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 1980 Nov 27;288(5789):373-6.

### 2.3.5 Using “We”

In a few biomedical fields, everyone uses “we” and in some other fields, no one does. However, in most fields, you or the journal editor may choose. Before you submit your article to the editor, skim the methods sections of several articles from a recent issue of the journal. Look for the use of active and passive verbs and follow the custom of the journal. If both are used, you may mix them.

Using the passive voice is more traditional, and some editors believe that using “we” is too informal. However, using the active voice (e.g. “we found that . . .”) is easier to read. It also draws attention to the authors. In the most competitive fields, authors are trying to find new ways to promote themselves, so many have started to use “we” more frequently.

**Tip**

When writing formally without co-authors, use “we” instead of “I.” In English, we sometimes call this “the royal ‘we’” because kings and queens once used it, or “the editorial ‘we’” because it is common in newspaper editorial opinions. You can also use it in conference presentations and for presenting yourself as a consultant in business.

### 2.3.6 Choosing Active or Passive in Other Situations

In the cases presented below, there are some guidelines, but there are no strict rules, so if the journal editor doesn’t have a preference, you can make your own decision about writing style. You may also wish to observe how other authors in your field handle these cases.

#### **Innovation**

According to Swales and Feak, “There is a tendency for passive sentences to indicate routine procedures while active sentences indicate new, deliberately chosen, important, or unexpected procedures.”

Swales JM and Feak CB, 1994 p. 161

Turner recommends: “Use active sentences using “we” or “our” if you want to emphasize YOUR decisions, YOUR method, or YOUR innovation. For standard procedures, generally use the passive structure.”

Turner A. *English Solutions for Engineering Research Writing*. 2006. p. 166.

In the following example, excluding subjects over 60 is presented as an important decision based on the results of a previous study.

**Example:**

Nyengaard and Bendtsen [14] observed that the number of glomeruli decreases with age owing to the accelerated loss of glomeruli after the age of 60 years. Consequently, **we excluded** all subjects who were 60 or older.

Keller G, Zimmer G, Mall G, Ritz E, Amann K. Nephron Number in Patients with Primary Hypertension *N Engl J Med* 2003;348:101-8.

## Emphasis

Also use active sentences any other time you want to emphasize a point. Here's an example from the same article that even uses the word "emphasize."

### Example:

Irrespective of the ongoing discussion concerning the optimal method of counting glomeruli, **we would emphasize** that in the present controlled study, the difference between the patients with hypertension and the control subjects was so large and consistent that it is highly unlikely that the result was due to a methodologic artifact.

## Broad Statement

When a journal allows the use of "we," it is also more common to describe the general method with the active voice at the beginning of the paragraph, and then use the passive voice for a more detailed description of the method in the rest of the paragraph.

### Example:

To estimate the number of glomeruli and the average glomerular volume per kidney, **we determined** the number of points on the grid that touched the cortical area, including the glomerular area; the number of points on the grid that touched the glomerular area; and the number of glomeruli found in the reference section. Cortical areas with obvious technical artifacts **were excluded**. The sampling volume **was calculated** by multiplying total tissue area (the number of points on the grid that touched the cortical area x the grid area) by the thickness of the section (e.g., 3  $\mu\text{m}$  x 8 sections = 24  $\mu\text{m}$ ). A correction for tissue shrinkage (x1.04) **was made**, and the resulting volume, multiplied by the specific weight of the fixed kidney, yielded the mass of the portion of the cortex being examined ( $m_{\text{exam cor}}$ ). The weight of the kidney under examination **was divided** by the weight of the total kidney cortex, yielding a ratio ( $m_{\text{exam cor}} : m_{\text{total cor}}$ ). The number of glomeruli **was then determined** with the following equation:  $\text{number} = 1 \div (m_{\text{exam cor}} : m_{\text{total cor}}) \times \Sigma Q$ , where Q- is the number of glomeruli found in the reference section but not in the comparison section.

Keller G, Zimmer G, Mall G, Ritz E, Amann K. Nephron Number in Patients with Primary Hypertension N Engl J Med 2003;348:101-8.

## 2.3.7 Using a Noun or Adjective instead of the Passive Verb

Many verbs can be changed to nouns or adjectives:

need  $\rightarrow$  necessary  
inform  $\rightarrow$  information  
fail  $\rightarrow$  failure  
propose  $\rightarrow$  proposal

Occasionally, a noun or adjective may communicate a point more clearly than a passive verb. In both examples below, the actor is not mentioned.

### Example:

The passive is needed.



The passive is necessary.

In the following example, two other options would be "We excluded/included" and "X, Y, and Z were excluded/included." The active voice is not necessary, as this is not an innovative or particularly interesting part of the procedure. The passive would be difficult to read because of the long list. Therefore, using "inclusion" and "exclusion" is the best option.

### Example:

**Inclusion criteria** comprised death before the age of 60 years; concentric left ventricular hypertrophy, a medical history of primary hypertension, or both; and the characteristic arteriolar lesions of the kidney found in patients with hypertension. **Exclusion criteria** were evidence of secondary hypertension, diabetes, a history of alcohol or drug abuse, or evidence of renal disease on histologic examination of the kidney.

Keller G, Zimmer G, Mall G, Ritz E, Amann K. Nephron Number in Patients with Primary Hypertension. N Engl J Med. 2003 Jan 9;348(2):101-8.

## 2.4 Purpose and Manner Expressions

In addition to simply describing your actions, the methods section includes justifications, or reasons why you carried out certain procedures. When the procedure is routine, explaining your goal may not be needed, but when the procedure is new, complex, or controversial, you should include a purpose statement to answer the question “Why?”

### 2.4.1 Purpose

A purpose statement explains the goal of an action.

In the examples, below . . .

A = action

B = goal

These three expressions follow the same pattern:

[Clause A] to [verb B].

[Clause A] in order to [verb B].

[Clause A] so as to [verb B].

#### Example:

The mixture is heated **so as to** separate the strands of DNA.

The order can also be switched:

To/In order to [verb B], [clause A].

#### Example:

**In order to** separate the strands of DNA, the mixture is heated.

#### Tip

“So as to” is rarely used at the beginning of a sentence and is less common in research reports. On the other hand, “to” and “in order to” are very common in the methods section of research reports and usually appear at the beginning of the sentence.

Consider the examples again.

#### Examples:

The mixture is heated **so as to** separate the strands of DNA.

**In order to** separate the strands of DNA, the mixture is heated.

In both cases, what is the goal?

Answer: *to separate the strands of DNA*

What is the procedure for reaching the goal?

Answer: *The mixture is heated.*

Here are two more purpose expressions. These ones use a gerund ( ), or “-ing” verb.

[Clause A] for the purpose of [verb+ing B].  
[Clause A] with the aim of [verb+ing B].

**Example:**

The mixture is heated **with the aim of** separating the strands of DNA.

One more purpose expression connects two clauses.

[Clause A] so that [clause B].

**Example:**

The mixture is heated **so that** the strands of DNA will separate.

## 2.4.2 Manner

A manner statement explains how to do something. It is the reverse of a purpose statement, and is also common in the methods section.

In the examples below . . .

A = action

B = manner

Here is the pattern:

[Clause A] by [verb+ing] [noun]

Purpose and manner statements are closely related. Compare these two sentences:

**Examples:**

The mixture was heated **in order to** separate the strands of DNA. (purpose)

The strands of DNA were separated **by heating** the mixture. (manner)

What was the goal (of heating the mixture)?

*to separate the strands of DNA*

How (were the strands separated)?

*by heating the mixture*

Basically, the information is the same, but the focus is different. The emphasis is on the first part of the sentence in each case.

|

## 2.5 Condensed and Extended Methods Descriptions

When your procedure is routine, the methods section may be brief, but when you develop a new procedure or use a controversial procedure, you will need to explain it in more detail. Most papers contain a mixture of routine and novel methods, so they will contain a mixture of brief and detailed descriptions.

According to Swales and Feak (2004), there are several differences between condensed (brief) and extended (detailed) descriptions of procedures. They include the two topics discussed above, purpose and manner statements, plus a few more:

### Variation in Methods Sections

Condensed	Extended
Tends to describe familiar, standard methods	Tends to describe new or unusual methods
Assumes background knowledge	Provides background information
No named subsections	Several named subsections
Uses abbreviations and citations as shorthand	Uses longer descriptions
Running series of verbs (e.g., “collected, stained, and stored”)	Usually one finite (main) verb per clause
Few “by + verb-ing” statements (explaining “how”)	Several “how” statements (see Manner above)
Few definitions and examples	More definitions and examples
Few justifications (explaining “why”)	Several justifications (often “In order to . . .”) (see Purpose above)
Few linking phrases	Wide range of linking phrases

Adapted from Swales JM, Feak CB. *Academic Writing for Graduate Students*, 2<sup>nd</sup> ed. Ann Arbor: U of Michigan; 2004.

These characteristics are usually mixed in a methods section, even in the same paragraph. The following example is typical.

## 2.5.1 Example of a methods section included in the article

The traditional research article contains a separate methods section after the introduction. In this typical example, aspects of both condensed and extended styles are mixed.

### Materials and Methods

Abbreviation

**Mice.** *PrlR1/2* mice were maintained on C57Bl6 3 129SV background. Genotyping for PrIR was carried out by **PCR** on tail DNA as described (9). Three-week-old F1 females of 129SV 3 C57Bl6 crosses were used as recipients. Their inguinal mammary glands were surgically cleared of the endogenous epithelium **as described** (12). The mice were mated 6 weeks after transplantation, and the engrafted glands were analyzed, together with control unmanipulated glands from the same mouse, by wholemount microscopy. For histological analysis they were subsequently embedded in paraffin and 8- $\mu$ m sections were cut and stained with hematoxylin and eosin or, alternatively, processed for immunohistochemistry as described (13).

Citation of a common or familiar method

**Mammary Gland Wholemounts.** The glands **were dissected, spread** onto a glass slide, **fixed** in a 1:3 mixture of glacial acetic acid/100% ethanol, **hydrated, stained** overnight in 0.2% carmine (Sigma) and 0.5% AlK(SO<sub>4</sub>)<sub>2</sub>, **dehydrated** in graded solutions of ethanol, and **cleared** in 1:2 benzyl alcohol/benzyl benzoate (Sigma). Pictures **were taken** on a Leica MZ12 stereoscope with Kodak Ektachrome 160T.

Running series of verbs

**Retroviral Supernatant.** The chimeric receptor prl-EpoR (CHI) was described (14). Prl-EpoR and PrIR were each modified **by the addition of three consecutive N-terminal FLAG epitopes inserted just after the signal sequence**. The addition of these FLAG epitopes did not affect receptor function (data not shown). Retroviral supernatants encoding either prl-EpoR or PrIR were generated as described (14). Briefly, VE23 cells were transiently transfected **by using the calcium phosphate method**, with MSCV retroviral constructs (15) each encoding the desired receptor. Culture supernatants were collected at 48 and 72 h after transfection and immediately frozen. Retroviral titers were determined **by infecting primary fetal liver cells with known dilutions of each retroviral supernatant**; 48 h posttransfection, expression of PrIR or prl-EpoR on the cell surface was determined by FACS analysis by using antibodies directed against the FLAG epitope. PrIR and prl-EpoR supernatants of similar titers were chosen for MEC infection.

Named subsections

A single main verb in the clause

Citation of a common or familiar method

Longer, more detailed description

**Cell Culture.** Primary mammary epithelial cells were prepared from 10-week-old virgin female mice as described (16). Primary cells were plated on collagen-coated dishes and maintained in DMEM/F12 with insulin (10 mg/ml) and EGF (5 ng/ml). The viral supernatants **were placed** on the mammary cells at day 3 of culture in the presence of 40 mg/ml polybrene and 5 ng/ml cholera toxin (17). One or 2 days later, cells were trypsinized and resuspended in PBS, and  $0.5 \times 10^6$  cells in a 100- $\mu$ l volume were injected into each cleared fat pad.

A single main verb in the clause

## 2.5.2 Example of a Methods Section at the End of the Article

A newer format for long journal articles with detailed methods involves putting most of the procedural details at the end of the article in a separate methods section and integrating the most important procedures into the results section—IRD(m) instead of IMRD. See the results chapter for another more detailed example.

### Part One: From the main text

Within the main article, the authors use concise language to explain their procedure. They may provide reasons, but not as many details.

### Example of Methods Sentence Embedded in the Results Section

## RESULTS

### Lack of PPs in $relB^{\pm/\pm}$ and $nfkB2^{\pm/\pm}$ mice

Histological examination of adult mice revealed that RelB is required for PP development. Whereas wild-type mice had several easily detectable PPs, serial sections of Swiss rolls of the small intestine did not reveal any histological evidence of rudimentary PPs in  $relB^{\pm/\pm}$  mice. While  $nfkB1^{\pm/\pm}$  mice had small PPs with a poorly developed microarchitecture,  $nfkB2^{\pm/\pm}$  mice also lacked PPs and only occasionally had lymphoid aggregates in the small intestine (data not shown). **To examine whether PP development is blocked at an early stage in  $nfkB2^{\pm/\pm}$  and  $relB^{\pm/\pm}$  mice, we stained whole intestines from newborn mice for VCAM-1.** Figure 1 shows that VCAM-1+ PP organizing centers formed normally in wild-type (Figure 1A) and  $nfkB1^{\pm/\pm}$  mice (Figure 1B), but were absent in  $nfkB2^{\pm/\pm}$  (Figure 1C) and  $relB^{\pm/\pm}$  animals (Figure 1D). Thus, both p52/p100 and RelB are essential for the development of PPs, whereas the p50 subunit of NF- $\kappa$ B plays only a minor role in this process.

### Part Two: From the methods section after the main article

At the end of the article, the authors provide details necessary for replicating the study. These details would not be interesting to most readers, but would be very useful for someone who wants to do a similar study. This part will include many citations that describe common or familiar methods. It will also explain new methods more extensively.

### Example of the Methods Section Describing the Same Procedure in More Detail

## MATERIALS AND METHODS

### Immunohistochemical analyses

Whole-mount immunohistochemistry was performed **as described previously** (Yokota et al., 1999). In brief, intestines **were fixed** in 2% paraformaldehyde overnight, **washed** with PBS and **subjected** to serial dehydration with methanol. **Following** 0.1% H<sub>2</sub>O<sub>2</sub> treatment and rehydration, non-specific binding was blocked with PBSMT [2% skimmed milk, 0.3% Triton X-100 in phosphate-buffered saline (PBS)] and specimens were incubated overnight with anti-VCAM-1 mAb (PharMingen; clone 429, diluted 1:1000). **After** washing in PBSMT and PBST (0.3% Triton X-100 in PBS) and incubation with anti-rat IgG-horseradish peroxidase (1:500), color reactions were performed using diaminobenzidine (DAB) and nickel chloride

Same procedure.  
Very different  
descriptions.

(Vector Laboratories). RelB immunohistochemistry was performed **as described previously** (Weih et al., 2001).

Yilmaz ZB, Weih DS, Sivakumar V, Weih F. RelB is required for Peyer's patch development: differential regulation of p52-RelB by lymphotoxin and TNF. *EMBO J.* 2003 Jan 2;22(1):121-30.

## 2.6 Replicable or Not

In some fields, giving enough information so that your readers could replicate your study is critical. In other fields, it is less important. It may also depend on the journal. When you're not sure whether to provide more detail, look at other articles in the journal you are aiming for. Often, the detail can be made available in supplements online.

## 2.7 Borrowing Routine Expressions

Beyond the purpose statements, there is typically little analysis in a methods section. It includes your procedures, but not the results or analysis of results. Therefore, much of the language of methods sections can be borrowed from other papers. It is acceptable to borrow sentences that describe routine procedures. However, if the procedure was reported for the first time by a certain author, you should cite that author's paper.

## 2.8 Describing Your Population

Most biomedical researchers do not concern themselves with differences among ethnic groups. However, in a few fields of study, such as nutrition, characteristics such as nationality, ethnicity, and/or religion may be relevant in the definition of the population studied. There are also some rare genetic illnesses that tend to appear in patients of a certain race or nationality. As a result, writers have to find the best way to describe these groups. The best way is often more specific and accurate than the way that we describe people in everyday conversation.

In Korea, the vast majority of the population fits into the same ethnic group and shares a similar cultural background. As a result, much of the clinical research simply specifies "in Korea" or "in Koreans" in the title and descriptions of the population studied. However, it is important not to make the same assumption about research carried out in other countries. If you cite a study that took place in Australia, for example, and no ethnic description is given, the correct description of the research subjects would be "patients from Australia" or "residents of Australia." There are many Koreans and other East Asians living in Australia. A few might be included in the research sample. Therefore, in a literature search for a disease that purportedly occurs more often in East Asians, it would not be correct to assume that the Australian study contains no East Asians, unless the authors have specifically mentioned so.

Likewise, the population of Korea is becoming more diverse. According to the Korean Ministry of Justice, there are now over a million foreign-born residents of Korea. Therefore, in describing your own research population, it should become standard practice soon to specify "ethnic Koreans" or "residents of Korea." The former would include people with Korean parents or grandparents living abroad. The latter would include anyone from any country living in Korea.

Korean Ministry of Justice Immigration Service. <http://www.immigration.go.kr/HP/IMM/index.do> . Go to "자료실" -> 통계자료실 -> 2007년 12월 통계월보. Retrieved on February 11, 2008.

According to Article Requirements section on "Defining Ethnicity" from the medical journal BMJ, "Ethnicity and culture are socially determined variables of limited use in biological research, though they are useful in health services research. All the variables are confounded by socioeconomic status."

The BMJ recommends that authors "try to use accurate descriptions of race, ethnicity, and culture rather than catch all terms in common use. In the methods section of research articles describe

the logic behind any ethnic groupings used.”

The BMJ goes on to list a number of ways to describe your sample, including:

- genetic differences
- self assigned ethnicity, using nationally agreed guidelines
- observer assigned ethnicity
- country or area of birth (participant's own, or parents' or grandparents' if applicable)
- years in country of residence
- religion.

BMJ Article Requirements. <http://resources.bmj.com/bmj/authors/article-submission/article-requirements> . Retrieved January 21, 2008.

The following example describes a study in Japan that includes residents of Japan who are from other countries or who have ancestors from other countries. Note how they are described.

### Example:

We recruited 11 unrelated patients with BCD and characteristic clinical features; **eight of Japanese, two of Middle Eastern, and one of Chinese ancestry**. . . Two novel mutations, L173W and Q450X, were identified in **a Japanese patient** and **two unrelated patients from Middle Eastern countries**, respectively.

Lin J, Nishiguchi KM, Nakamura M, Dryja TP, Berson EL, Miyake Y. Recessive mutations in the CYP4V2 gene in East Asian and Middle Eastern patients with Bietti crystalline corneoretinal dystrophy. J Med Genet. 2005 Jun;42(6):e38..

