QUALITY ASSURANCE OF VITAL® TEST MEAL IN GASTRIC EMPTYING SCINTIGRAPHY

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QUALITY ASSURANCE OF VITAL® TEST MEAL IN GASTRIC EMPTYING SCINTIGRAPHY

by

NGO HUI WEN

Thesis submitted in fulfilment of the requirements
for the degree of
Bachelor of Health Science (Honours) (Medical Radiation)

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CERTIFICATE

This is to certify that the dissertation entitled "QUALITY ASSURANCE OF VITAL® TEST MEAL IN GASTRIC EMPTYING SCINTIGRAPHY" is the bona fide record of research work done by Ms "NGO HUI WEN" during the period from October 2023 to July 2024 under our supervision. We have read this dissertation and that in our opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Health Science (Honours) (Medical Radiation).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

C	Counts
BOT	Bottom
$\%$	Percentage
P	Phytate
^{99m}Tc	Technetium 99-metastable
TOP	Top

LIST OF ABBREVIATIONS

ANMS	American Neurogastroenterology and Motility Society
AGF	Aspirated gastric fluid
EWM	Egg-white meal
GI	Gastrointestinal
GE	Gastric emptying
GES	Gastric Emptying Scintigraphy
GM	Geometric mean
OGDS	Oesophago-Gastro-Duodenoscopy
QA	Quality assurance
ROI	Region of interest
SPECT/CT	Single Photon Emission Computed Tomography/Computed Tomography
SNM	Society of Nuclear Medicine
SD	Standard deviation

LIST OF APPENDICES

JAMINAN KUALITI TERHADAP VITAL® UNTUK SINTIGRAFI PENGOSONGAN PERUT

ABSTRAK

Kajian in vivo terhadap sintigrafi pengosongan gastrik (GES) telah menunjukkan bahawa minuman nutrient berkalori tinggi, Vital®, boleh digunakan sebagai alternatif bagi individu yang tidak boleh mengambil hidangan putih telur pepejal konvensional (EWM). Penilaian lanjut diperlukan untuk memastikan kebolehpercayaannya sebagai makanan cecair untuk GES. Kajian ini menujukkan dua aspek jaminan kualiti untuk mengesahkan Vital®, termasuk penyebaran seragam radiofarmaseutikal dalam minuman nutrien dengan kecekapan and kestabilan perlabelan radiofarmaseutikal dalam vitro. **Kaedah:** Satu keadah penyediaan yang mudah telah dicadangkan di mana ^{99m}Tc-phytate (^{99m}Tc-P) disuntik ke dalam minuman tanpa membuka kertas aluminium, dan botol itu digoncang perlahan selama satu minit. Minuman itu kemudian diimejkan selama 15 minit menggunakan sintigrafi dinamik, dan analisis kawasan minat (ROI) di bahagian atas dan bawah botol dilakukan. Untuk eksperimen kecekapan and kestabilan radioperlabelan, 12 sampel Vital® dicampur dengan ^{99m}Tc-P, diinkubasi dengan supernatan air liur dan cecair gastrik manusia selama 4 jam, kemudian disentrifugasi dan ditapis setiap jam. Radioaktiviti pada bahagian pepejal dan penapis dinilai menggunakan pengimejan statik selama 1 minit. **Keputusan:** Hasil kajian menunjukkan tiada perbezaan yang ketara antara radioaktiviti antara bahagian atas dan bawah botol selama 15 minit ($p > 0.05$), menunjukkan penyebaran seragam radiofarmaseutikal dalam minuman nutrien. Lebih banyak radioaktiviti ditemui dalam filtrat berbanding bahagian pepejal ($p < 0.05$), menunjukkan radiopelabelan yang lebih keutamaan pada fasa akueus Vital®. Radiofarmaseutikal kekal stabil dalam kedua-dua bahagian pepejal dan akueus Vital®

dalam persekitaran gastrik selama 4 jam, dengan tiada perbezaan yang ketara diperhatikan antara setiap titik masa ($p > 0.05$). **Kesimpulan:** Kajian ini memberikan informasi yang lebih mendalam tentang bagaimana minuman nutrien Vital® yang bertinggi kalori berkelakuan, meningkatkan pemahaman tentang penemuan GES sebelumnya. Ia boleh menjadi rujukan utama untuk penyelidikan dan amalan klinikal masa depan dalam memilih dan menyediakan hidangan ujian cecair, yang dapat membawa kepada keputusan GES yang lebih tepat.

QUALITY ASSURANCE OF VITAL® TEST MEAL FOR GASTRIC EMPTYING SCINTIGRAPHY

ABSTRACT

In vivo studies of gastric emptying scintigraphy (GES) had demonstrated that the high-calorie drink, Vital® could serve as an alternative to people who cannot tolerate the conventional solid egg-white meal (EWM). Further evaluations were needed to ensure its reliability as a liquid meal for GES. Therefore, we sought to address two quality assurance aspects to validate Vital®, which included uniform dispersion of the radiotracer and in vitro radiolabelling efficiency and stability. **Methods:** A simple mixing method was proposed where ^{99m}Tc-phytate (^{99m}Tc-P) was injected into the Vital® drink without removing the aluminium seal, and it was gently swirled for a minute. The drink was then imaged for 15 minutes using dynamic scintigraphy and an analysis of the regions of interest (ROI) at the top and bottom of the of the drink was done. For the radiolabelling efficiency and stability experiment, 12 samples of Vital® were mixed with ^{99m}Tc-P, incubated with human saliva supernatant and gastric fluid for 4 hours, then centrifuged and filtered at hourly intervals. The radioactivity of the solid part and filtrate was assessed using 1-minute static imaging. **Findings:** Results showed no significant differences in activity percentages between the top and bottom of the drink over 15 minutes ($p > 0.05$), suggesting a uniform distribution of the radiotracer throughout the drink. Significantly more radioactivity was found in the filtrate compared to the solid part ($p < 0.05$), indicating a preferential radiolabelling of the aqueous phase of Vital®. The radiotracer remained stable in both the solid and aqueous phases of Vital® in the simulated gastric environment over 2-4 hours, with no significant differences observed between each time point ($p > 0.05$). **Conclusions:** This study offered detailed insights into how the high-

calorie Vital® nutrient drink behaves, improving the understanding of previous GES findings. It could serve as a key reference for future research and clinical practices in choosing and preparing liquid test meals, leading to more accurate GES results.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Gastric emptying scintigraphy (GES) is a valuable, non-invasive diagnostic technique that is used to assess and manage patients with non-specific upper gastrointestinal (GI) symptoms such as pain, early satiety, postprandial fullness, bloating, nausea, and vomiting (Wise *et al.*, 2020). The primary goal of this imaging procedure is to quantify the rate at which a test meal empties from the stomach into the small intestine, categorizing it as normal, delayed, or accelerated (Farrell, 2019; Nawi *et al.*, 2020).

GES is a procedure where the patients ingest a standard solid meal, typically a low-dose (1.0 mCi) radiolabelled egg-white sandwich meal (EWM), and then undergoes serial scanning with a gamma camera at specific time points. The gamma camera captures the radiation emitted by the radiotracer in the meal and forms a planar image showing the radioactivity distribution within the body. The radioactivity in the stomach is thus directly proportional to the amount of food present in the stomach at any given time.

The EWM is endorsed by the American Neurogastroenterology and Motility Society (ANMS) and the Society of Nuclear Medicine (SNM) as the gold standard for GES (Abell *et al.*, 2008). However, some patients are incapable of ingesting the prescribed test meal due to various factors like egg or gluten allergies, dietary restrictions, difficulty with solid foods, or personal lifestyle preferences. In such cases, the appropriate action is to cancel the GES procedure (Wise *et al.*, 2021). Other critiques of EWM include

the inconsistent standardized practices among agencies regarding ingredient quantities and cooking styles, alongside the time-consuming preparation process (Tagiling *et al.*, 2024).

In the United States, a high-calorie liquid meal such as Ensure® Plus is commonly used as an alternative to EWM (Solnes *et al.*, 2019). However, this product is not currently retailed in Malaysia. A recent study conducted by researchers in Malaysia explored the use of Vital®, a high-calorie liquid drink with similar caloric density (1.5 kcal/mL) and nearly equivalent nutrient composition to Ensure® Plus (Tagiling *et al.*, 2024). The randomized crossover trial study involving healthy participants, demonstrated that the emptying rates of Vital® were comparable to those of EWM, particularly for the late stages of gastric emptying (GE). This finding suggested that Vital® could be a useful option for patients (Tagiling *et al.*, 2024). Additionally, it was a convenient and easily accessible meal choice that required no cooking or extensive preparation.

While the in vivo findings are promising, this high-calorie liquid test meal's quality assurance (QA) has yet to be determined. Therefore, this study seeks to build upon the previously mentioned clinical trial by investigating the QA of Vital® for GES, focusing on pre-ingestion radiotracer uniformity in the drink as well as in vitro radiolabelling efficiency and stability in gastric conditions.

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1.2 Problem statement

The reliability of a GES can be influenced by the thorough mixing of the radiotracer throughout the test meal (Bonta *et al.*, 2014) as well as the efficient and stable binding of the radiotracer to the test meal (Somasundaram *et al.*, 2014). The counts measured in the stomach by scintigraphy directly correspond to the volume of the ingested meal in the stomach (Abell *et al.*, 2008). This correlation underscores the importance of proper mixing during meal preparation and stable labelling of the test meal with the radionuclide to ensure an accurate GES interpretation.

Allergies and vegetarianism among patients pose challenges when preparing the EWM for GES (Solnes *et al.*, 2019). According to a survey by Wise *et al.* (2020), when a patient has an egg allergy, 31% of institutions modify the test by substituting the egg with a different material, while 52% use an entirely different type of meal. These findings, in turn, indicated that various alternative GE meals were in use. Several alternative solid GE meals, such as cheddar cheese (Drubach *et al.*, 2010; Liu *et al.*, 2016), instant oatmeal (Liu *et al.*, 2016; Pal *et al.*, 2022), and rice cakes (Pal *et al.*, 2022; Somasundaram *et al.*, 2014), have been examined for their in vitro radiolabelling efficiency and stability.

Despite growing evidence suggesting that the GE of a high-calorie nutrient liquid meal was comparable to that of the standardized EWM, at least in healthy individuals (Sachdeva *et al.*, 2013; Solnes *et al.*, 2019; Tagiling *et al.*, 2024), none of these studies examined the QA of the test meal. This omission challenges the validity of the in vivo findings. Hence, it is essential to fill this gap to ensure a level of confidence in the in vivo results.

1.3 Research questions

1. Does the radiotracer disperse uniformly within the Vital® drink prior to consumption?
2. How is the labelling efficiency of radiotracer to the Vital® in a gastric environment over 4 hours?
3. Does the Vital® have a preferential radiolabelling phase (i.e., aqueous or solid)?
4. How is the labelling stability of radiotracer to the Vital® in a gastric environment over 4 hours?

1.4 Research objectives

1.4.1 General objective

To assess the quality assurance of the Vital® as an alternative test meal for GES.

1.4.2 Specific objectives

1. To determine the uniformity dispersion of radiotracer within the Vital® drink prior to consumption.
2. To determine the labelling efficiency of radiotracer to the Vital® in a gastric environment over 4 hours using in vitro static digestion model.
3. To determine the labelling stability of radiotracer to the Vital® in a gastric environment over 4 hours using in vitro static digestion model.

1.5 Research hypotheses

1.5.1 Null hypothesis, H_0

1. H_0: There is no uniform dispersion of radiotracer within the Vital® drink prior to consumption.
2. H_0: There is no significance difference in the labelling efficiency of radiotracer to the solid and aqueous phase of Vital® in a gastric environment over 4 hours.
3. H_0: The labelling of radiotracer to Vital® was not stable in a gastric environment over 4 hours.

1.5.2 Alternative hypothesis, H_1

1. H_1: There is no uniform dispersion of radiotracer within the Vital® drink prior to consumption.
2. H_1: There is a significance difference in the labelling efficiency of radiotracer to the solid and aqueous phase of Vital® in a gastric environment over 4 hours.
3. H_1: The labelling of radiotracer to Vital® was stable in a gastric environment over 4 hours.

1.6 Significance of study

The findings of this study are useful in developing an alternative liquid meal protocol that is more robust, convenient and suitable for more patients, while also being easier for staff to prepare. This study can offer valuable insights for researchers and healthcare professionals concerning the QA of the liquid meal, including the thoroughness of mixing radioactivity in the test meal before consumption and, the in vitro radiolabeling efficiency and stability of a liquid meal in a gastric environment. Without a clear understanding of the characteristics of the liquid test meal, the reliability of the established in vivo GES study could be compromised.

In our opinion, Vital® is a viable alternative GES meal as it can be prescribed to patients with specific dietary needs and GI dysfunction. Moreover, it comes pre-packaged with a standardized formula and is widely available in various countries, eliminating the need for meal preparation. This study could serve as a baseline for future work with patients and be useful for establishing normative GE values for alternative meals in other populations.

CHAPTER 2

LITERATURE REVIEW

2.1 Anatomy of stomach

The human stomach, located beneath the diaphragm, is a distended digestive system component with four distinct radiological anatomical shapes during fasting: the steer-horn, cascade, J-shape and fishhook (Figure 2.1) (Miftahof, 2017). It connects the oesophagus to the duodenum and comprise four primary sections: cardia, fundus, body and pyloric part (Chaudhry *et al.*, 2022). The cardia is where the oesophagus and the stomach meet, while the fundus is a dome-shaped structure. The body is the largest central portion of the stomach, while the pyloric part is further divided into three regions: the pyloric antrum, pyloric canal, and pylorus (Figure 2.2) (Soybel, 2005). The pyloric sphincter establishes a border between the stomach and the duodenum.

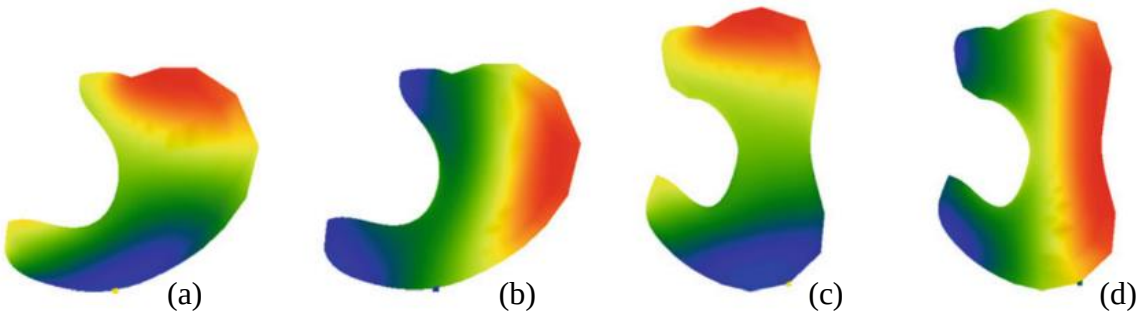

Figure 2.1: Four distinct radiological anatomical shapes of human stomach during fasting: (a) Steer-horn, (b) Cascade, (c) J-shape, (d) Fishhook (Miftahof, 2017).

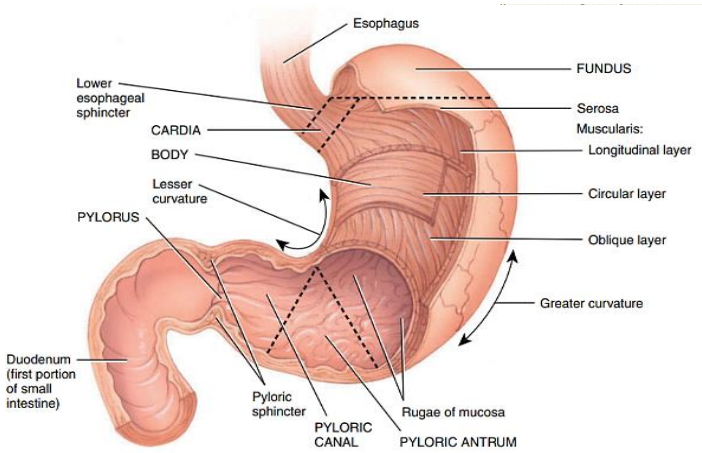

Figure 2.2: Anatomy of stomach (Soybel, 2005).

2.2 Gastric digestion

2.2.1 Mechanical digestion

After passing through the oesophagus, the bolus enters the stomach, where it undergoes mechanical and chemical digestion. Mechanical digestion occurs through peristaltic contractions of the smooth muscle, propelling the bolus from the fundus toward the antrum (Patricia & Dhamoon, 2022). The fundus primarily serves as storage with fewer contractions, while stronger contractions start in the stomach body and intensify near the antrum (Rogers, 2011; Sensoy, 2021). The antrum forcefully pushes the bolus against the pyloric sphincter, grinding it to reduce food particle size. The pyloric sphincter only allows particles smaller than 2 mm to pass into the duodenum, while larger particles are moved back into the stomach for further digestion. This cyclic process of propulsion, grinding, and retropulsion thoroughly mixes gastric contents with gastric juice, forming a liquified substance called chyme (Goyal *et al.*, 2019; Sensoy, 2021).

2.2.2 Chemical digestion

Chemical digestion in the stomach is driven by gastric juices secreted by glands in the stomach lining. These juices contain hydrochloric acid, pepsin, lipase, mucus, intrinsic factor, and other substances essential for nutrient absorption (Tortora & Derrickson, 2014). The acidity of HCl kills microorganisms and activates pepsin which is crucial for protein digestion. Pepsin is secreted as an inactive pepsinogen and is activated by H+ ions in gastric secretions (Blanco & Blanco, 2017). The gastric environment is highly acidic, with a pH typically between 1 and 3 (Singh, 2019). Pepsin is most active at a pH of 2, retains 70% of its maximum activity at pH 4.5, and shows minimal activity at pH 5.5 (Gray *et al.*, 2014). Lipid digestion in the stomach is limited as gastric lipase functions best at a pH of 5-6 (Tortora & Derrickson, 2014).

2.3 Gastric emptying (GE)

When the food particles in chyme become sufficiently small, they can pass through the pyloric sphincter, a process known as gastric emptying (GE) (Jacoby, 2017). Within 2 to 4 hours post-meal, the stomach empties its contents into the duodenum

2.3.1 Solid GE study

When solids are ingested, the process of GE typically follows a biphasic pattern (Hellström *et al.*, 2006). Initially, there is a lag phase corresponding to a solid trituration phase lasting 30 to 60 minutes (Hellström *et al.*, 2006). During this time, the stomach redistributes solid foods from the fundus to the antrum and processes them into 1-2 mm particles (Farrell, 2019; Hellström *et al.*, 2006). Following the lag phase, there is an

equilibrium emptying phase called the linear emptying phase, during which the stomach gradually releases the digestible solids through the pylorus into the small intestine (Liu *et al.*, 2020).

2.3.2 Liquid GE study

GE of liquids is simpler because they can distribute rapidly throughout the stomach and pass through the pyloric sphincter without requiring trituration (Banks *et al.*, 2023; Farrell, 2019). The emptying process of different caloric liquid solutions is varied. Low-caloric liquids, such as water, tend to empty from the stomach more rapidly than high-caloric liquids, which are often more nutrient-dense and energy-rich. Emptying non-nutrient or low-caloric liquids is a mono-exponential process with an initial fast phase followed by a slower late linear phase (González *et al.*, 2000; Liu *et al.*, 2020).

On the other hand, high-calorie liquid may display a lag phase during gastric emptying, contributing to a more prolonged presence in the stomach (Liu *et al.*, 2020). Goyal *et al.* (2019) found significant differences in GE times between water and high-calorie liquid. They discovered that around 50% of the ingested water had left the stomach within 10 minutes, while 50% of the high-calorie liquid remained for up to 2 hours after ingestion (Goyal *et al.*, 2019).

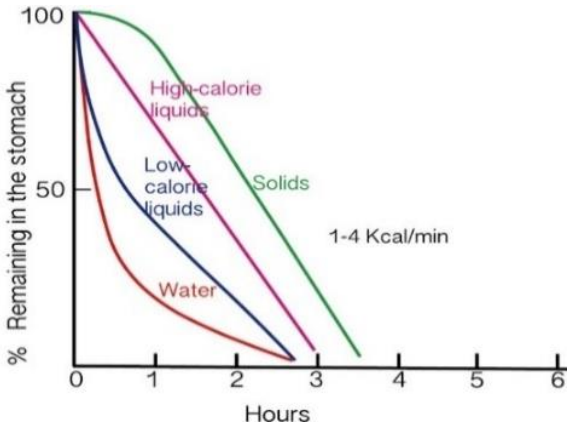

Figure 2.3: Emptying of different states of food (Goyal *et al.*, 2019).

2.4 Gastric emptying scintigraphy (GES)

As of today, GES remains the gold standard for assessing gastric emptying and/or motility due to its physiological, non-invasive, and quantitative nature (Farrell, 2019). In 2008, the ANMS and the SNM issued recommendations to standardize the methodology. They recommended using a low-fat, technetium-99m (^{99m}Tc) labeled EWM, with imaging at 0, 1, 2, and 4 hours after meal ingestion (Abell *et al.*, 2008). GES measures the normal, delayed, or accelerated rate at which the stomach empties (Farrell, 2019). The published normal solid meal GE values were at 30 minutes, ≥ 70% of the meal should be retained; at 1 hour, 30% - 90% should be retained; at 2 hours, no more than 60%; and at 4 hours, no more than 10% (Abell *et al.*, 2008). Gastric retention > 60% at 2 hours or > 10% at 4 hours suggested delayed GE, while a retained meal value < 70% at 30 minutes or 30% at 1 hour indicated rapid GE. Data suggested that a 4-hour imaging period was more sensitive for detecting abnormal emptying than 2 hours (Ziessman *et al.*, 2007).

2.4.1 Analysis of GES

Assessing the images alone is insufficient for determining whether gastric emptying is rapid or delayed (Farrell, 2019). To measure how well the stomach empties, specific regions of interest (ROIs) are delineated around the stomach in images acquired, encompassing both the antrum and fundus (Farrell, 2019). The stomach lies obliquely within the abdomen, with the fundus situated more posteriorly and the antrum more anteriorly. When the food moves from the fundus to the antrum, there is an apparent increase in counts in the anterior region due to reduced depth (Chiappin *et al.*, 2007). In other words, the gastric counts obtained from imaging may be influenced by the variation in depth and directional movement of ingested food within the stomach (Farrell, 2019). Therefore, correcting for attenuation when measuring the gastric counts obtained from the ROIs for each time point is necessary.

The most used method for attenuation correction is the geometric mean (GM) approach (Farrell, 2019; Seok, 2011). GM is the square root of the product of anterior counts (C_{ANT}) and posterior counts (C_{POST}), as shown in equation (1) below:

$$Geometric\ mean\ = \sqrt{(C_{ANT}\ \times\ C_{POST})} \quad (1)$$

GM is preferably determined from anterior-posterior data acquired simultaneously with a dual-head gamma camera. The GM, corrected for decay, is then utilized to calculate the percentage (%) of activity retained (food remaining) in the stomach at specific intervals. This methodology is achieved by dividing the total counts observed at each time interval by the initial total counts (Farrell, 2019). Subsequently, the percentage of gastric activity is plotted against time.

2.5 Challenges associated with current consensus standardized GE test meal

The standard GE test meal consists of 120 g of liquid egg white (160 kcal) mixed with ^{99m}Tc-sodium colloids (^{99m}Tc-SC) and served with white bread (120 kcal), 30 g of strawberry jam (75 kcal) and 120 ml of water (Abell *et al.*, 2008).

Figure 2.4: Consensus standardized EWM.

However, a common clinical challenge arises when many patients cannot or refuse to consume the EWM used for medical or diagnostic purposes. Reasons for this contraindication include allergies to ingredients like eggs or gluten, difficulties eating solid foods, adherence to dietary preferences, or religious beliefs that conflict with the meal's contents (Sachdeva *et al.*, 2013). In addition, recent surveys found that many Asian institutions still do not properly follow the standardized EWM protocol, even though the guideline was published almost 20 years ago (Tagiling *et al.*, 2024). Reasons for this include the complex preparation of EWM, which involved many components and specific cooking methods, and a lack of awareness about the guidelines. This variation in EWM practices might explain the differences between dyspeptic symptoms and GES results (Tagiling *et al.*, 2024).

The challenges above highlight the need for a simpler, more straightforward standardised alternative GE test meal. High-calorie nutrient drinks are likely one of the most viable options for alternative GE test meals.

2.6 High-calorie nutrient drinks

To date, three in vivo GES studies have assessed the potential of high-calorie nutrient drinks (~1.5 kcal/ml) as a replacement for the EWS. To further illustrate, Sachdeva *et al.* (2013) and Solnes *et al.* (2019) compared Ensure® Plus with the EWM in healthy volunteers. They found that the stomach emptied at similar rates for both meals, supporting Ensure® Plus as a viable alternative. Similarly, Tagiling *et al.* (2024) compared Vital® nutrient drink with the standardized EWM. They found that the liquid meal resulted in faster early emptying but had a comparable amount remaining at the 4-hour mark, suggesting its suitability as an alternative.

As per the European Society for Clinical Nutrition and Metabolism guidelines, standard energy formulas typically range from 0.9-1.2 kcal/ml, with high-energy formulas exceeding this range and low-energy formulas falling below (Lochs *et al.*, 2006). The high-calorie nutrient drinks used in the studies mentioned above have a calorie value of 1.5 kcal/ml and are ready-to-drink. One serving of Ensure® Plus (237 ml) contains 350 kcal while one serving of Vital® (200 ml) provides 300 kcal. Both nutritional supplements have nearly similar nutritional profiles (Tagiling *et al.*, 2024).

Figure 2.5: Abbott Vital® nutrient drink.

2.7 Quality assurance of GE test meal

Generally, quality assurance (QA) often refers to quality management that provides confidence that quality requirements will be fulfilled (Manghani, 2011). In the context of this study, the QA of the GE test meal was proposed as a proactive process primarily concerned with ensuring the reliability of the in vivo GES study. The proposed QA consisted of two main aspects: the uniformity of the radiotracer in the test meal prior to ingestion as well as the radiolabelling efficiency and stability of the radiotracer to the test meal in gastric conditions.

2.7.1 Uniformity of radiotracer dispersion in test meal before ingestion

Proper mixing of the radiotracer to the test meal is essential to ensure an accurate GE measurement. For egg-whites, properly mixing (cris-cros) using a fork inside a casserole for at least 1 minute ensures a good uniform radiolabelling and potentially minimizes false-negative GES studies (Bonta *et al.*, 2014). While the proper cooking of egg whites to a firm consistency can be easily checked by visual inspection, it is difficult

to evaluate the uniform distribution of radioactivity in the prepared (Bonta *et al.*, 2014), not to mention in liquid drinks. There is currently no established method for mixing liquid test meals. Therefore, we proposed a simple mixing method within the same time frame of at least 1 minute.

2.7.2 Radiolabelling efficiency and stability of test meal

An ideal GES test meal should have a radiolabel that remains stable within the meal and is not absorbed by or adhered to the GI mucosa (Knight, 2012). To ensure a valid GES study, the radiotracer binding to the meal is of utmost importance. The radiotracer must stay associated with the phase it is supposed to represent during exposure to gastric fluid, whether it is a solid or liquid test meal. Hence, every new GE meal must be evaluated for stability in gastric fluid in vitro (Knight, 2012).

Several in vitro radiolabelling efficiency and stability studies have been conducted to explore other solid test meals. For instance, it was observed that the chapatti and rice cake labelled with ^{99m}Tc-SC demonstrated higher labelling stability when compared to bread & butter and oatmeal and can be alternatives to egg whites for vegetarians or those allergic to eggs (Pal *et al.*, 2022). This study confirmed an earlier investigation by Somasundaram *et al.* (2014), which suggested that a radiolabelled rice cake can be used as an alternative solid meal for routine GES studies. Cheddar cheese was also seen as an alternative to egg-whites for paediatric GES, as it maintained a high degree of radiolabelling stability (95.6 ± 1.0 %) and is well-liked by children (Drubach *et al.*, 2010).

On the other hand, the existing literature for liquid-based test meals are somewhat limited. The three in vivo studies mentioned above did not demonstrate the in vitro

radiolabelling efficiency and stability of high-calorie nutrient drinks. Meanwhile, another two in vitro studies by Liu *et al.* (2016) and Hooda *et al.*, (2018) found that the standard calorie Ensure (~1.08 kcal/mL) has a radiolabelling stability that ranges between ~70% to ~80% from 2 hours to 4 hours. The two studies used different radiotracers: one used ^{99m}Tc labelled diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA), while the other used ^{99m}Tc alone. The radiolabelling stability was notably lower when compared to the results on solid-based test meals. To the best of our knowledge, no studies have been conducted to evaluate the radiolabelling efficiency and stability of high-calorie nutrient drinks despite the fact that they are the only liquid test meal deemed fit to substitute for a solid GE test meal. Addressing this gap is crucial to ensure the reliability of findings that align with those observed in in vivo settings.

Ensure® Plus and Vital®, like other homogenized oral nutritional supplement beverage, can be separated into three general phases (Figure 2.6): lipid phase, aqueous phase, and pellet phase (Klein, 2009). Understanding whether the radiotracer would be present in the aqueous, lipid, or pellet phase could offer valuable insights into the emptying dynamics of the high-calorie liquid drink.

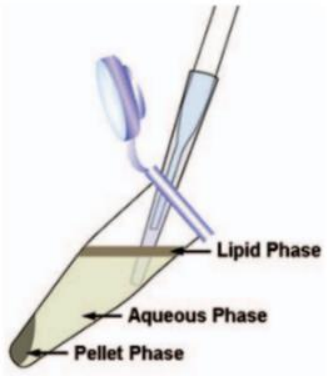

Figure 2.6: Post-centrifugation of Ensure® Plus sample resulted in phase separation (Klein, 2009).

2.8 In vitro static gastrointestinal (GI) model

In vitro methods that simulate digestion processes, are commonly employed to study how food or pharmaceuticals behave in the GI tract (Minekus *et al.*, 2014). These methods offer several advantages such as being more cost-effective, less time-consuming, requiring less labour, and not being subject to ethical restrictions (Minekus *et al.*, 2014). At present, the most widely used method is a static in vitro digestion model developed by the INFOGEST international consortium (Zhou *et al.*, 2023). The model typically simulates the oral, gastric, and small intestinal phases of digestion, aiming to replicate physiological conditions observed in vivo (Brodkorb *et al.*, 2019). This dissertation focused on replicating the static phases of oral and gastric digestion, following the protocol Minekus *et al.* (2014) described.

2.8.1 Oral phase

To maintain consistency with dilution factors, it is recommended to include an oral phase in digestion simulations for both solid and liquid foods (Mulet-Cabero *et al.*, 2020). 1: 1 (v/v) of food with simulated salivary fluid is targeted, followed by a 2-minute incubation in a shaking water bath at 37°C (Brodkorb *et al.*, 2019; Minekus *et al.*, 2014).

2.8.2 Gastric phase

Liquid food can undergo an optional oral phase or be directly exposed to the gastric phase; otherwise, it is compulsory for solids (Minekus *et al.*, 2014). In the gastric phase, two digestive processes, mechanical and chemical, are replicated (McKee *et al.*, 2019). Mechanical digestion is typically mimicked using a shaking water bath (Liu *et al.*, 2020),

while chemical digestion involves creating an acidic environment with a pH level similar to the stomach's natural acidity. During this phase, the oral bolus is mixed with simulated gastric fluid at a 1:1 ratio, and digestion is recommended to occur for 2 hours at 37℃ (Minekus *et al.*, 2014; Brodkorb *et al.*, 2019).

2.9 Conclusion

To summarise, ^{99m}Tc-P was used for radiolabelling the Vital® in this study. A simple proper mixing was introduced to evaluate the thorough distribution of radioactivity within the Vital® bottle. Besides, the radiolabelling efficiency and stability of Vital® were examined in gastric environment over 4 hours using an in vitro static digestion model. Human saliva and gastric fluid were used instead of simulated fluids since they were readily available in our research setting and better approximate the human condition.

CHAPTER 3

METHODOLOGY

3.1 Study design and ethical clearance

This prospective study was designed following an experimental quantitative approach. The study involved collecting the saliva and aspirated gastric fluid (AGF) from human subjects. Before commencing the study, ethical approval was sought and obtained from the Human Research Ethics Committee (JEPeM) of Universiti Sains Malaysia (USM) under the protocol code USM/JEPeM/KK/24010022 (APPENDIX B). Human subjects voluntarily participated in specimen collection after being informed about the research. Their consent was documented through signed forms provided in the APPENDIX C. No conflicts of interest that could influence the impartiality of this study were declared. Subject identities were kept confidential and only accessible to the research team. Specimens were anonymized and destroyed at the study's end. The study adhered to the ethical principles of the Declaration of Helsinki and the Malaysia Good Clinical Practice Guidelines.

3.2 Study area

This study took place at Hospital USM, Kubang Kerian, Kelantan. The AGF was collected at the Endoscopy Unit, while the experiments were carried out at the Central Research Lab (CRL) of the School of Medical Sciences and the Nuclear Medicine Department of Hospital USM.

3.3 Study population

This study collected the fresh saliva from volunteers to simulate the oral phase of digestion. The volunteers were required to be healthy and over 18 years old, while those who had received antibiotic treatment within the last 3 months were excluded. Written informed consent was obtained from the volunteers.

On the other hand, AGF was collected from patients scheduled for Oesophago-Gastro-Duodenoscopy (OGDS) at Hospital USM from March - May 2024 (3 months). Individual informed written consent was obtained from those who gave permission for the collection. The inclusion criteria included patients aged over 18 years old, scheduled for elective OGDS from the Surgical Outpatient Department (SOPD). The exclusion criteria included patients scheduled for emergency OGDS or from the ward. The AGF specimens collected with a pH of less than 4 were included for simulating the gastric digestion phase while those with a pH above 4 were excluded.

3.4 Sample size estimation

This study did not employ formal sample size calculations due to the nature of the experimental study. For assessing the radiolabelling efficiency and stability, 12 samples of 1 ml Vital® were used. To simulate the oral phase of digestion, 1 ml of saliva was added to each sample, necessitating a total of 12 ml of saliva, which two to three healthy volunteers could provide. About 2 ml of AGF was added to each sample for the gastric digestion phase, requiring a total of 24 ml. If 2 ml of AGF could be obtained per patient, around twelve patients would be needed to meet the volume requirement. However, this estimate was contingent on the pH being less than 4 during subsequent pH testing.

3.5 Sampling method

Convenience sampling was used for saliva and AGF collection in this study. This sampling entailed selecting individuals primarily because they were readily available or easily accessible to the researchers. In this approach, the principal investigator recruited healthy volunteers from the campus compound through word of mouth and approached the patients scheduled for OGDS at the Endoscopy Unit of Hospital USM.

3.6 Study materials

Table 3.1: List of materials used in this study.

Materials	**Quantity**
^{99m}Tc-phytate	1 mCi
Instant thin-layer chromatography strip	1
Dose calibrator	1
Vital®	4
Human saliva	12 ml
Suction trap	3
Human gastric fluid	24 ml
pH meter	1
pH buffer solutions (pH 7.00, pH 4.01, pH 10.01)	1
Disposable pipette 3 ml / Micropipette 1000 µl	1
Centrifuge tube 15 ml	36
Plastic test tube rack	2

Benchtop centrifuge	1
Ultracentrifuge seal tube 5 ml	2
Ultracentrifuge	1
Incubator shaker	1
Beaker 250 ml	2
Sterile gauze pack	1
Syringe 5 ml	12
Isotonic saline	24 ml
Dual-head gamma camera	1
Xeleris™ 3.1 Workstation	1
Distilled water	500 ml
75% alcohol swab pack/spray	1
Reusable ice pack	4
Cooler box	1
Freezer -80°C	1

A variety of materials were utilized in this study, as outlined in Table 3.1. The ^{99m}Tc-phytate (Technephyte; Center of Molecular Research, Russia) was a radiotracer (radiopharmaceutical) used to label the test meal in this study. ^{99m}Tc-P was a feasible alternative to the gold standard, ^{99m}Tc-SC, for routine in vivo GES (Nawi *et al.*, 2020). It was more affordable due to the lower price tag of the phytate kit. The synthesis of ^{99m}Tc-P was also simpler as the phytate kits did not require the incubation procedures with ^{99m}Tc, which are necessary for kits such as nano-colloid and DTPA (Nawi *et al.*, 2020).

Before the test meal was prepared, the quality control test on the ^{99m}Tc-P, was performed by a radio-pharmacist using an instant thin-layer chromatography (ITLC) strip (Tec-Control™ Chromatography Strips; Biodex Medical Systems). This step was a standard procedure commonly practised in clinical settings to ensure the safe and effective use of the radiotracer, intending to achieve a radiochemical purity level of >95%.

Vital® (Abbott Laboratories, Malaysia) (Figure 2.5), a 200 ml high-calorie nutrient drink, was used as the test meal throughout this study. One serving of Vital® provided 300 kcal with 36.8 g carbohydrate (49%), 13.5 g protein (18%), and 11 g fat (33%). This nutrient drink was fibre-free, gluten-free, suitable for lactose intolerance, kosher, and halal.

A dose calibrator (AtomLab™ 500; Biodex Medical Systems) (Figure 3.1) was used to measure the radioactivity of ^{99m}Tc-P before adding it to the test meal. The dose calibrator was a well-type, cylindrical ionization chamber used in nuclear medicine to measure the activity of radioactive doses administered to patients. Filled with pressurized argon gas, it measured radiation in the millicurie (mCi) range. Inside the chamber, the collecting electrode captured ions produced by radiation from radiopharmaceutical interaction with gas. This ion measurement corresponded to the activity of the radiopharmaceutical dose.

Figure 3.1: AtomLab™ 500 dose calibrator.