*Review Article*

# Challenges in Production of Alzheimer's Tracer C-11 PiB



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**Abstract**
Beta-amyloid plaques and neurofibrillary tangles have been known as the neuropathological hallmarks of Alzheimer's disease. $^{11}$C Pittsburgh Compound B (PiB) ([C-11]PiB) is the first successful and well-studied radiopharmaceutical for Positron Emission Tomography (PET) imaging of beta-amyloid deposition in the brain. Although [C-11]PiB has been used in western countries since 2002, Wattanosoth Hospital was the only center with the capacity to produce [C-11]PiB in South-East Asia in 2012. This article describes briefly the challenges the PET radiopharmaceutical team at Wattanosoth Hospital have been facing in the production of [C-11]PiB. It also provides a brief protocol used here for production of [C-11]PiB.

Alzheimer's is the most common form of dementia. According to the Alzheimer's Association, Alzheimer's disease accounts for an estimated 60 percent of dementia cases.[1] It also reported that about one in nine people aged 65 and older (11%) have Alzheimer's disease and about one-third of people aged 85 and older (32%) have Alzheimer's disease. According to the National Center for Health Statistics, Alzheimer's disease is listed as the sixth-leading cause of death in the United States.[2] Early clinical symptoms for Alzheimer's disease include: difficulty remembering names and recent events, apathy and depression.[1] Later symptoms include impaired judgment, disorientation, confusion, mood and behavior changes and difficulty in speaking, swallowing and walking. Although Alzheimer's disease is not curable at present, many researchers believe that future treatments to slow or stop Alzheimer's disease will be most effective when administered during pre-clinical and mild cognitive impairment (MCI) stages of the disease.

The brain pathological findings of Alzheimer's disease patients are deposits of protein fragment beta-amyloid (plaques) and twisted strands of the protein tau (tangles) as well as evidence of nerve cell damage and death in the brain.[1] The definitive confirmation of Alzheimer's disease is by postmortem histopathological examination of beta-amyloid deposits in the brain. Therefore, beta-amyloid in the brain is one of the useful biomarkers for differential diagnosis of Alzheimer's disease. Biomarkers can be used to indicate the earliest signs of disease even though symptoms such as memory loss have not yet developed. Many researchers believe that Alzheimer's-related brain changes may begin 20 years or more before symptoms occur. In 2011, the National Institute on Aging (NIA) and the Alzheimer's Association proposed new criteria and guidelines for diagnosing Alzheimer's diseases.[1,3-6] The new criteria propose that Alzheimer's disease begins before the development of symptoms, and that new technologies have the potential to identify brain change before the development of symptoms. The new criteria and guidelines identify two biomarker categories: (1) biomarkers showing the level of beta-amyloid accumulation in the brain and (2) biomarkers showing that neurons in the brain are injured or actually degenerating.


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Beta-amyloid imaging provides in vivo detection of fibrillar beta-amyloid (Aβ) plagues of Alzheimer's disease.[7] $^{11}$C Pittsburgh Compound B (PiB) is the most well studied amyloid imaging agent.[7-10] It is a radiotracer for imaging amyloid plaques using Position Emission Tomography (PET). [C-11]PiB binds with high affinity to aggregated synthetic beta-amyloid fibrils. The in-vivo retention of [C-11]PiB in brains of people with Alzheimer's disease shows a regional distribution that is very similar to the distribution of beta-amyloid deposits in post-mortem studies.[7] Compared to age-matched normal controls, Alzheimer's disease patients have 2-3 folds greater [C-11]PiB retention on PET scans in brain areas.[8,11] Many studies have shown that [C-11]PiB has a very high sensitivity of 90% for detecting beta-amyloid in patients with Alzheimer's disease.[12-15]

**"Pittsburgh Compound B"** or [C-11]PiB or PiB for short is also known as [$^{11}$C]6-OH-BTA-1 or (N-Methyl-[$^{11}$C])2-(4'-methylamino-phenyl)-6-hydroxy-benzothiazole. It was developed by a research team from the University of Pittsburgh led by the geriatric psychiatrist William E. Klunk and the radiochemist Chester A. Mathis.[16,17] [C-11]PiB is a radioactive analog of thioflavin T which was modified to include C-11 isotope. Thioflavin T is a fluorescent dye used by pathologists to identify plaques in brain tissue specimens. [C-11]PiB has high affinity to amyloid and can penetrate across the blood brain barrier with rapid clearance from background areas for maximum visualization of amyloid pathology. Through a collaboration with a researcher team in Uppsala University in Uppsala, Sweden, the radiopharmaceutical labeled with C-11 was first used in human studies in 2002.[8] The name **"Pittsburgh Compound B (PiB)"** was given its name by the Swedish researcher team from Uppsala University.[10]

**Challenges for production of [C-11]PiB**

I. **The first challenge** is the high energy gamma ray and short half-life of C-11. C-11 decays by positron emission. It results in 511 keV gamma rays. The radiochemists work with very high levels of penetrating radiation. The half-life of C-11 is 20 minutes which is very short. Therefore, a large amount of starting radioactivity is needed. To synthesise [C-11]PiB, the starting activity of C-11 at Wattanosoth Hospital is more than 2 Ci. This results in a potentially high radiation dose to radiochemists. Thus to minimize radiation exposure, everything has to take place in a hot cell. The synthesis of [C-11]PiB has been done by using automation/remote manipulation. Since, the radioactivity is reduced by 50% every 20 minutes; every step must be fast, simple and reproducible. Production must be coordinated with the PET scan. The quality control of the product has to be fast as well. With a 20 minute half-life for C-11, the C-11 radiopharmaceuticals cannot be transported long distances. Normally, the PET exam with C-11 radiopharmaceutical needs an in-house cyclotron.

II. **The starting material** (C-11) is a gas. It needs careful handling to avoid contamination and unintentional release into the facility. A good gas handling system for waste gas is needed to avoid any radioactive gas leak or contamination of the facility.

III. **Specific activity** (SA) is a major concern. Specific activity of a radiopharmaceutical is the amount of radioactivity per unit mass of a compound. In the case of the specific activity of [C-11]PiB, the specific activity is the activity of [C-11]PiB divided by the mass (or molar amount) of the sum of all radioactive and stable nuclides present in the same chemical and physical form. In short, it is the ratio of radioactivity to cold mass. The contamination of atmospheric $CO_2$ will decrease the specific activity of C-11 radiopharmaceutical significantly. The maximum theoretical specific activity of C-11 radiopharmaceutical is about 9,257 Ci/μmol. The practical specific activity of C-11 labeled radiotracers is considerably lower. Practical specific activity rarely exceeds 100 Ci/μmol and in typical practice it is about 1-10 Ci/μmol. With a C-11 labeled radiotracer having a specific activity of 1 Ci/μmol, roughly only 1 in 10,000 tracer molecules contain C-11, with the remaining containing mostly C-12.

Generally, the radiopharmaceuticals that bind to the molecular target e.g. neuroreceptors, transporters protein, and enzymes, high specific activity is required. For the radiopharmaceuticals that trace in vivo processes such as metabolism or blood flow, high specific activity is not required. Most receptor based studies require a minimum of 500 mCi/μmol at the end of synthesis in order to maintain the tracer principle. The recommendation for specific activity of [C-11]PiB is more than 300 mCi/μmol at the time of administration. The specific activity of C-11 radiopharmaceutical varies greatly among various production facilities. There are numerous factors affecting the specific activity of C-11 radiopharmaceuticals. Those factors can be grouped into 3 groups, namely the starting amount of radioactivity, the amount of C-12 contamination, and the amount of time in which it takes to do the chemistry.

The starting amount of radioactivity depends on the choice of cyclotron and targets, and the yield of the radiochemistry. For example, for 10 MeV protons and 17 MeV protons, the yield of C-11 production is almost triple at higher energy.[18] Therefore, with the same bombardment time; one can make more activity of C-11 with higher energy than the lower energy. The chemistry method with faster and higher radiochemical yields obviously produces higher radioactivity.




**Figure 1:** C-11 PRO2 autosynthesis module in a hot cell for [C-11]PiB synthesis at Wattanosoth Hospital.[11]

The amount of C-12 contamination is another important issue. It depends on many factors. Cyclotron targets, target body material, foils, physical target sizes and volumes also affect the specific activity. The purity of the target gas is another issue. Even with the highest purity nitrogen (UHP, 99.9999%) and the use of a hydrocarbon trap, there is still a trace of C-12. The presence of carbon dioxide ($CO_2$) from the air containing 98.88% of C-12 and 1.12% of C-13 compete with C-11]$CO_2$ produced from the cyclotron. Besides carbon carrier contamination in the target gas, the source of carbon contamination can come from carbon absorbed in the target chamber, $CO_2$ penetrating in the production line and $CO_2$ absorbed in the lithium aluminum hydride (LAH) solution. LAH solution could become a major source of $CO_2$. LAH will absorb $CO_2$ and will affect specific activity. High quality LAH is essential for a high specific activity product.

## Production of [C-11]PiB at Wattanosoth Hospital

The production of [C-11]PiB at Wattanosoth Hospital is performed by using iPHASE C-11 PRO-2 automated C-11 radiolabelling module. C-11 PRO-2 is a dual reactor C-11 radiolabelling lab which can produce a vast variety of C-11 radiotracers. Figure 1 shows the C-11 PRO-2 module in the hot cell at Wattanosoth Hospital.

[C-11]PiB at Wattanosoth Hospital is produced by a Grignard reaction using 6-OH-BTA-0 precursor (2-(4'-aminophenyl)-6-hydroxybenzothiazole; GMP grade).

## C-11 Production and labeling of [C-11[PiB

C-11[$CO_2$] is produced by TR-19 PET cyclotron using nuclear reaction $^{14}N(p,\alpha)^{11}C$. TR-19 PET cyclotron is a particle accelerator producing up to 19 MeV proton beam manufactured by Advanced Cyclotron System INC (ACSI). To produce C-11, 0.1% oxygen in nitrogen with 99.9999% purity is used as a target gas. The target gas is irradiated with 19 MeV protons. The C-11 species that is formed reacts readily with the small amount of oxygen in the target gas. The primary precursors C-11 carbon dioxide ([C-11]$CO_2$) are formed.

After the irradiation is finished, [C-11]$CO_2$ is transferred from the cyclotron to the C-11 PRO-2 automate synthesis module in the hot cell. The [C-11]$CO_2$ is trapped on a molecular sieve and then it is delivered to a solution of lithium aluminum hydride (LAH) in tetrahydrofuran (THF) in a reaction vial. C-11 methyl iodide ([C-11]$CH_3I$) is produced using the "wet method" of lithium aluminum hydride (LAH) followed by hydriodic acid (HI). Then [C-11]$CH_3I$ is converted to C-11 methyl triflate ([C-11]$CH_3OTf$) by passing through the silver triflate furnace and subsequently trapped in the high performance liquid chromatography (HPLC) loop. The [C-11]PiB is produced by labeling reaction of [C-11]$CH_3OTf$ and the precursor (6-OH-BTA-0) in the HPLC loop. The crude reaction mixture is then purified using HPLC purification for good separation. The radiochemical yield is about 18%-20% (decay corrected) with a total synthesis time


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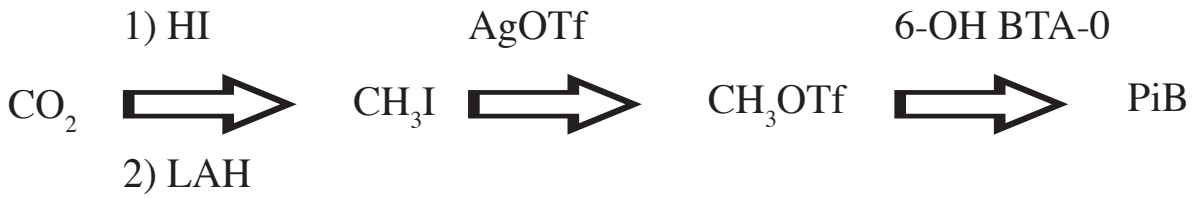



**Figure 2:** Main chemical reaction for [C-11]PiB synthesis

of about 30 minutes after receiving [C-11]$CO_2$ from the cyclotron. In routine synthesis at Wattanosoth Hospital, the starting activity of [C-11]$CO_2$, measuring at the molecular sieve, is about 1.8-2.3 Ci. [C-11]PiB produced at the end of synthesis is about 200-250 mCi. The specific activity of [C-11]PiB at the end of synthesis is about 2-5 Ci/μmol. The brief equation for radiosynthesis of [C-11]PiB is shown in Figure 2.

In order to minimize the presence of C-12 along the process of irradiation, transferring and synthesis of radiopharmaceuticals labeled with C-11, the following method is applied at Wattanosoth Hospital.

*For [C-11]$CO_2$ Production*

1. Use highest purity nitrogen (99.9999%) containing 0.1% oxygen as target gas.
2. Use Stainless Steel type 316 for transferring line and make sure that there is no leak.
3. Cold cleaning by flushing the target gas holder and transferring line with target gas for 10 minutes before pre-irradiation.
4. Hot cleaning by pre-irradiation for 5 minutes and dump to waste 5 times.
5. Pressurized transferring line with target gas during irradiation and after finishing irradiation.
6. Use the optimum irradiation time to produce highest C-11 yield with highest specific activity result. Several experiments have been done in order to find the optimum irradiation time. Currently, the irradiation time at Wattanosoth Hospital is 30 minutes with beam current of approximately 35 μA. With such condition, the amount of [C-11]$CO_2$ trapped in molecular sieve before synthesis is about 1.8-2.3 Ci.

*For [C-11]PiB Production*

1. Use highest purity nitrogen (99.9999%) with PiB synthesis module.
2. Heat molecular sieve and flow nitrogen gas pass through the molecular sieve before synthesis.
3. Use commercially-available LAH solution in glass vials closed with crown caps directly without further dilution or purification.
4. After the first use, the LAH solution is kept under helium in order to avoid contact with air. The solution will be transferred to the synthesis unit only a few minutes before the end of bombardment.

When starting the production here, the initial specific activity of [C-11]PiB was less than 300 mCi/μmol. Searching for an improvement of specific activity from extensive literature and expert's opinions along with experimenting with a lot of factors, finally the specific activity of [C-11]PiB was improved greatly. After applying the above protocol, the specific activity of [C-11]PiB becomes more than 2.5 Ci/μmol. Since the technical details are beyond the scope of this article, these details can be found in upcoming articles from our group. [C-11]PiB PET imaging in Wattanosoth hospital has been servicing patients with problems with dementia since November 2012. The PET images for [C-11]PiB for normal control and Alzheimer's patients are shown in Figures 3 and 4.

## Conclusion

Early detection of Alzheimer's disease is a key goal of current research to slow the progression of the disease. At present, the onset of Alzheimer's disease cannot yet be stopped or reversed. However, an early diagnosis allows patients with dementia and their families a better chance of benefiting from treatment, chances to participate in clinical drug trials and more time to plan for the future. With the effort to improve the quality of radiopharmaceuticals, the current [C-11]PiB production at Wattanosoth Hospital can service 2-3 patients a day requiring a PET examination.

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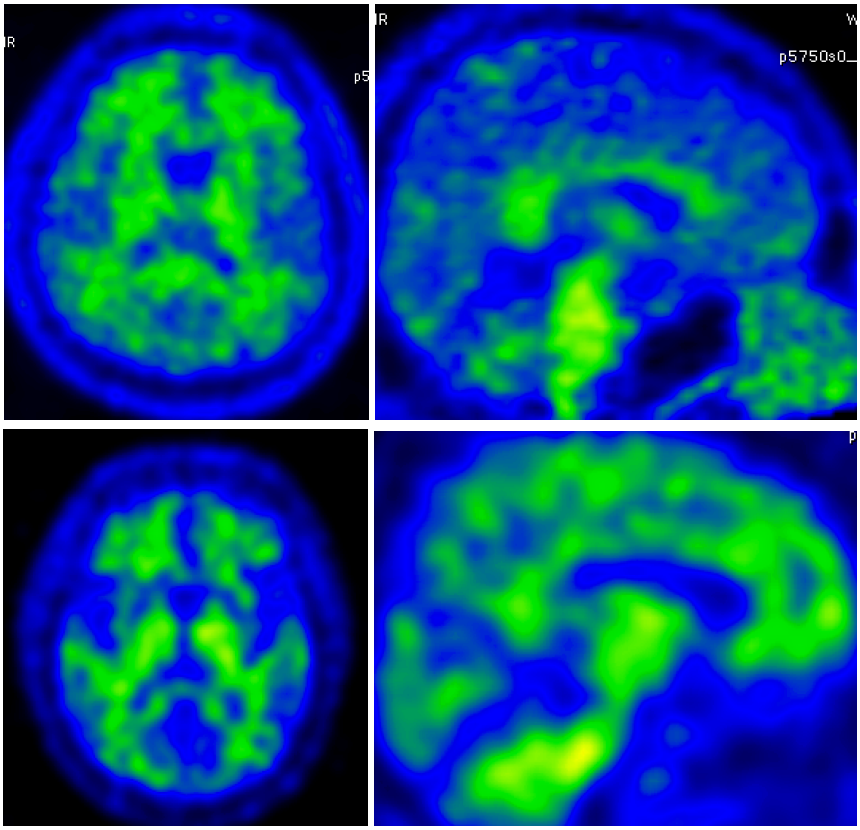


**Figure 3:** PiB-PET image in normal subject shows no accumulate of beta-amyloid over the cortex area.

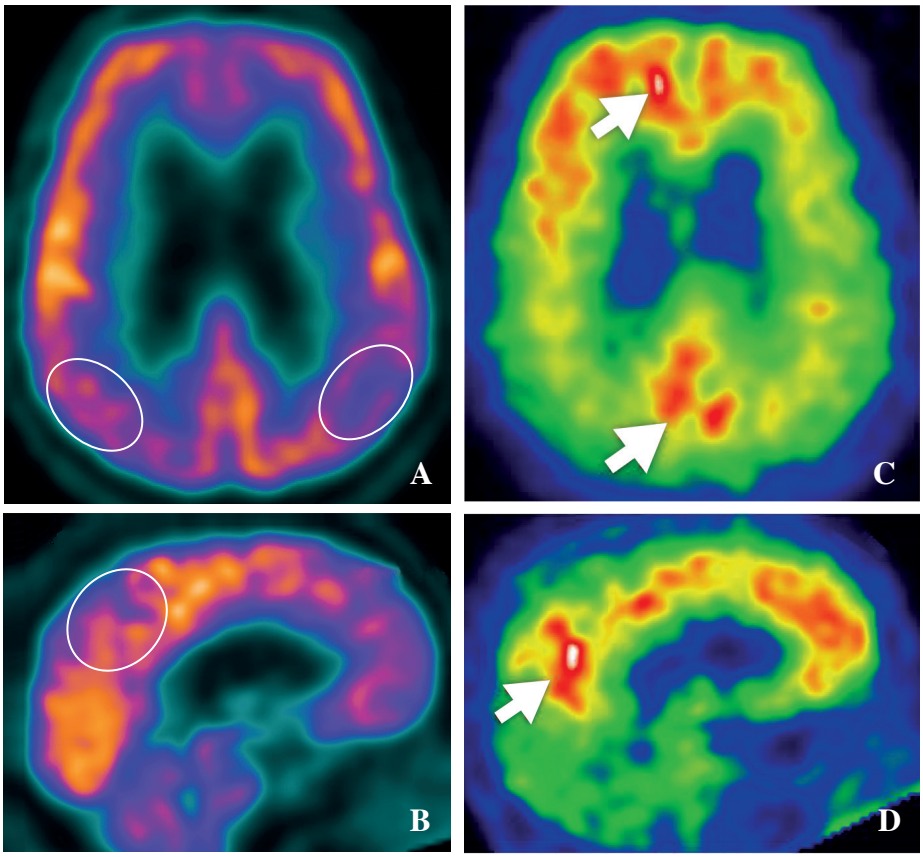



**Figure 4:** FDG-PET and PiB-PET image in Alzheimer's dementia patient. The FDG-PET reveals hypometabolic activity over bilateral parietal lobe, posterior cingulate and precuneus (A, B), whereas PiB-PET shows accumulation of beta-amyloid over the cortex areas (C, D).


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