

## Annex 4

### Pricing guidelines for external service viroid testing by SHQ Lab, WorldVeg 世界蔬菜中心種子健康檢疫實驗室 種子類病毒檢測服務收費規則

(限台灣地區 For Taiwan Only)

#### 世界蔬菜中心簡介

#### World Vegetable Center Introduction

The World Vegetable Center (WorldVeg) is a not-for-profit, autonomous International Agricultural Research Center with headquarters in Taiwan and regional offices in Africa and Asia. The Center mobilizes resources from the public and private sectors to develop and disseminate improved lines and production methods in developing countries. We help farmers make their vegetable production more intensive, efficient and sustainable. This increases vegetable harvests, creates jobs, and raises incomes in poor rural and urban households. It also provides healthier, more nutritious diets for families and communities.

世界蔬菜中心為非營利性國際農業研究機構，總部設立於台灣，另有分部設立於非洲與亞洲各地。集結公部門與私部門的資源，研發並推廣優良蔬菜品種及耕作方式至開發中國家。期望藉此協助農民進行集約、高效及永續耕作，以增加蔬菜產量，增加就業機會，改善農村或城市中貧困家庭的收入，並提供家庭及社區更加健康與營養的飲食。

WorldVeg holds the world's largest collection of vegetable germplasm in the public domain. The Center implements vegetable breeding programs in Asia and Africa, has strong connections to the seed sector, and is well placed to develop improved cultivars with traits for tolerance to biotic and abiotic stresses, enhanced shelf life, and improved nutrient content and quality.

亞蔬中心擁有全球公部門最大的蔬菜種原庫，在亞洲及非洲地區都有蔬菜育種的研究計畫，並與種子產業有強力的連結，具有強大的實力發展優良品系以抵抗生物性與非生物性逆境、延長的儲運期、改良的養分及品質。

#### 種子健康檢疫實驗室簡介

#### SHQ Lab at a glance

2014 年底，世界蔬菜中心在總部成立了種子健康檢疫實驗室。2015 年，種子健康檢疫實驗室建立了以反轉錄聚合酶連鎖反應(RT-PCR)檢測類病毒(Pospiviroids)的方法(由農業試驗所鄭櫻慧博士協助)。初期，種子健康檢疫室專注於茄科種子和植株的檢測，以及測試種子處理去除類病毒的方法；現階段亦逐步發展其他種子病害相關檢測。

World Vegetable Center established the Seed Health and Quarantine (SHQ) Lab at its headquarters in 2014. In 2015, a reverse-transcriptase polymerase chain reaction (RT-PCR) method for detection of Pospiviroids (viroids) was established in SHQ Lab with assistance from Dr. Ying-Huey Cheng, Taiwan Agricultural Research Institute (TARI). Initially, SHQ Lab focused on the detection of viroids in Solanaceous seeds and plants, and the testing methods for treating Solanaceous seeds to clean them of viroid contamination. Now the lab is extending its capacity to also test for other seed-borne diseases.

為服務國內農研產業，本中心對台灣地區合作夥伴如研究機關、種子公司、農民等提供種子及種苗的類病毒檢測服務。本服務將收取工本費用，以維持實驗室運作(非以營利為目的)。由於本中心 SHQ 實驗室尚未得到防檢局官方認證，其**檢測報告效力，僅供作為品質控管，尚**

無法提供行政院農業委員會動植物防疫檢疫局開立檢疫證明效力。

To support the Taiwanese horticultural sector, WorldVeg offers the service of testing seeds and seedlings for the presence of viroids and potentially other seed-borne diseases, e.g. Tomato brown rugose fruit virus, to research organizations, seed companies and farmers in Taiwan. For such service, WorldVeg will charge the requester the actual costs of conducting the test (not for profit). Since the SHQ lab is not a formally accredited testing facility, the test report is for quality control only, and cannot be used for the issuance or endorsement of a Phytosanitary Certificate by BAPHIQ.

### 服務項目 Service :

標的作物 Target crop : 茄科 Solanaceous crops

標的組織 Target tissue : 種子或葉片 seed or leaf

檢測病原 Target pathogens : 類病毒(馬鈴薯紡錘形塊莖類病毒、番茄黃色矮化類病毒、辣椒小果類病毒、番茄頂矮化類病毒、番茄類病毒及金魚藤潛伏類病毒) viroids (*Potato spindle tuber viroid* (PSTVd), *Tomato chlorotic dwarf viroid* (TCDVd), *Pepper chat fruit viroid* (PCFVd), *Tomato apical stunt viroid* (TASVd), *Tomato planta macho viroid* (TPMVd) and *Columnnea latent viroid* (CLVd))

### 申請方式 Application : (圖一)(Fig. 1)

填寫申請表，寄至 [seedhealth.quarantine@worldveg.org](mailto:seedhealth.quarantine@worldveg.org)，並電洽經辦人討論，世界蔬菜中心將會提供估價單及預估取得結果的時間，雙方取得共識後再討論如何進行樣品取樣。

Please fill in the request form, send it by e-mail to [seedhealth.quarantine@worldveg.org](mailto:seedhealth.quarantine@worldveg.org). WorldVeg staff will provide a price quote and an estimated time for results to be made available. Once the requester agrees on the price and timeline, methods of sending the samples can be discussed.

申請表 request form : 如附件一 (see attachment 1)

聯絡窗口 Contact information :

姓名 Name : xxx

電話 Telephone : xxx-xxxx

電子郵件 E-mail : xxx@xxxxxx

### Sampling methods to be done by requesters

#### A. 種子樣品取樣 Seed Sampling :

The basic unit of a seed sample

- Tomato/pepper/eggplant: at least 400 seeds or 1g (whichever is greater)

(If the seed number meets the requirement but still less than 1 g, please increase the seed amount to 1 g). We recommend the sample quantity to be at least 1% (by weight) of the seed lot. If one basic unit sample size does not satisfy 1% sampling rate of the seed lot, additional basic unit(s) of seed sample should be taken. The seed samples should not be pooled from multiple seed lots. For example:

- Seed lot size < 100g: 1 sample
- 100g < Seed lot size < 200g: 2 samples
- 200g < Seed lot size < 300g: 3 samples
- Seed lot size > 300g: follow the rules described above.

種子樣品：每一個單位樣品的基本種子量為：

- 番茄/番椒/茄子：400 粒種子(且≥1 公克)

(種子已達粒數但重量不足 1 公克時，請增加至 1 公克)

本中心建議取樣比例應達到該批號(seed lot)重量的百分之一以上。當 1 個基本種子量樣品未超過百分之一取樣比例時，則需再增加一個樣品，依此類推。不同批號的種子不應混合成為組合樣品。例如：

- 種子批重量 < 100 公克: 1 個樣品
- 100 公克 < 種子批重量 < 200 公克: 2 個樣品
- 200 公克 < 種子批重量 < 300 公克: 3 個樣品
- 隨種子批重量增加，樣品數依此類推。

種子樣品自行隨機取樣種子裝於小紙袋或鋁箔袋中，其上清楚標示送檢單位、樣品編號、植物普通名、學名等資訊(如圖二)。之後再以封口袋密封，裝於信封或紙箱以常溫寄送或親送。

收件地址：台南市 74151 善化區益民寮 60 號 世界蔬菜中心 種子健康檢疫實驗室收

Seed sampling: Please collect seeds randomly per seed lot. Seal the seed sample in a small paper or aluminum bag, and put it into a ziplock bag. Clearly mark the bags with information of the requester affiliation, sample number, sample common name, and scientific name.

Mail or deliver in person to the address: Seed Health and Quarantine Laboratory, World Vegetable Center, 60 Yi-Min Liao, Shanhua, Tainan 74151, Taiwan.

## **B. 葉片樣品取樣 Leaf Sampling：**

當要檢驗的植株生長至約 4 片展開的真葉大小時，採取第 3 與第 4 片已展開的真葉(建議同一個品系採取 10%的植株數目)，將同一品系的葉片樣品裝在一個塑膠袋中(合併樣品，最多一次合併 18 株)並標示送檢單位、樣品編號、植物普通名、學名、樣品編號等資訊(如圖二)。若不同植株要分開檢測，則各自裝於單獨的塑膠袋中。將採好葉片樣品的塑膠袋裝於紙箱中，以冷藏宅急便(箱內請勿放入保冷劑)寄到前述送件地址。為確保樣品在第一時間進行處理，請於週一至週三之間採樣，並預留週四及週五給本實驗室進行樣品處理與核酸萃取等檢測流程。

When the targeted plants grow to 4 fully expanded leaf stages, take the 3rd and 4th true leaves as sample. Recommended sampling rate is 10% of the total plant number. When taking samples from single plants, put the leaf samples from each plant in separate bags. When taking pooled samples from multiple plants of the same varieties/lines, please put leaves of the same variety/line in one plastic bag (max samples from 18 plants per bag). Clearly mark the bags with information of the requester affiliation, sample common name, scientific name and sample number. Put the sample bags in a cardboard box and send to the address listed above by refrigerated delivery (4°C, DO NOT put refrigerant packs inside the sample box). To ensure that the sample will be handled in a timely manner, please send it during Monday to Wednesday so SHQ Lab can receive the samples on Thursday or Friday to process them.

## **檢測方法 Technical information：**

反轉錄聚合酶鏈鎖反應檢測

(Reverse transcription polymerase chain reaction, RT-PCR)

本檢測法針對 Pospiviroid 類病毒進行檢測。利用種子研磨法及 3 組引子包括 Posp1(F)/Posp1(R) (Verhoeven et al., 2004)、Vd6up/ Vd4dw-2 (本技術授權由行政院農業委員會農業試驗所鄭櫻慧博士提供)、mtF2/ mtR1 (Lee and Chang, 2006)進行檢測。其中 mtF2/ mtR1 為植株

常態表現之核糖核酸 RNA 偵測引子對，為比對 RNA 萃取效能之引子對)。RT-PCR 反應完成後，以電泳分析核酸增幅產物所呈現之條帶位置，進行結果判讀。

The detection targets of this RT-PCR are Pospiviroids.

Primer pairs used for the detection:

- Vd6up/Vd4dw-2 for detection of *Columnea latent viroid* (CLVd) [technology transfer from Dr. Ying-Huey Cheng, TARI, Council of Agriculture (COA), Executive Yuan, Taiwan];
- Pospi1(F)/Pospi1(R) for detection of *Potato spindle tuber viroid* (PSTVd), *Pepper chat fruit viroid* (PCFVd), *Tomato apical stunt viroid* (TASVd), *Tomato chlorotic dwarf viroid* (TCDVd) and *Tomato planta macho viroid* (TPMVd) (Verhoeven et al, 2004).
- mtF2/ mtR1 amplify the plant *nad5* mRNA are used as internal control to monitor the RNA extraction efficiency.

The RT-PCR products are analyzed by gel electrophoresis.

## 收費規則 Pricing guidelines

### Pricing for viroid testing

檢測種子類病毒收費規則如下:

1. Viroid testing is performed by RT-PCR. Fee for each testing is calculated according to the number of samples to be tested. (Table 1)以反轉錄聚合酶連鎖反應法(RT-PCR)檢測，每批次依樣品數量收取樣品檢測費用，收費規則如表一。
2. This pricing for viroid tests is subject to adjustment according to price fluctuation of the supplies. Changes on pricing will be announced after official review and approval.  
以上收費規則依物價狀況不定期審查後公告調整。
3. Special conditions not included in this guideline will need further negotiation between SHQ Lab and the requester.  
若有本規則未列舉之特殊狀況，將由 SHQ 實驗室與委託單位另行約定。

Table 1. Pricing guideline for different sample types.

表一、收費規則。

Type 樣品種類	Unit 計價單位	Fee (NT\$) 費用(新台幣)
Handling fee 手續費	per batch of samples 每批樣品收費一次	300
Seed sample 種子樣品	per one sample 每一個樣品	1,500
Seed sample (same seed lot) 種子樣品(同一批號)	2 samples 2 個樣品	2,900

Seed sample (same seed lot) 種子樣品(同一批號)	3 samples 3 個樣品	4,200
Leaf sample 葉片樣品	per pooled sample or single sample 每一個組合樣品或單一樣品	1,500

#### Payment options

##### 付費方式：

\*The testing will start when the fee is fully paid and received by WorldVeg. 檢測將於付款完成後開始進行。

##### 1. 支票付款：

使用台灣地區的銀行所發行的支票，受款人寫上「亞洲蔬菜研究發展中心」，掛號郵寄到：台南市 74199 善化郵政第 42 號信箱

Check: Please mail a check drawn on a local Taiwan bank and payable to "Asian Vegetable Research and Development Center" to:

World Vegetable Center  
P.O. Box 42  
Shanhua, Tainan 74199  
Taiwan

##### 2. 郵局轉帳、匯款 Wire transfer:

行庫代號 Bank Code : 700

郵局帳戶名稱：亞洲蔬菜研究發展中心

Account Name: Asian Vegetable Research and Development Center

存簿帳號 Account Number : 0191457-0002504

轉帳、匯款手續費需自行負擔。請提供轉帳畫面照片或匯款單掃描檔，以電子郵件寄到 seedhealth.quarantine@worldveg.org 以方便對帳。

The banking fee of wire transfer is to be borne by the requester. The screen shot of wire transfer or a scanned copy of the payment voucher should be sent to WorldVeg via email to the following address: seedhealth.quarantine@worldveg.org, for our record.

##### 3. 請提供收據資訊：

Please provide the following information for SHQ Lab to issue a receipt of payment:

收據抬頭：Institute name or requester's name

統一編號：Company Tax ID

聯絡人：Contact name

寄送地址：Mail address

連絡電話：Phone number

電子郵件：E-mail address

#### 檢測報告 Reporting：

檢測所需時間：2-6 週

完成檢測後，檢測報告及收據會以掛號郵寄方式寄送至申請表單上留存的地址。

Testing time needed: 2-6 weeks

When tests are finished, the report and receipt will be sent to the address listed on the request form by registered mail.

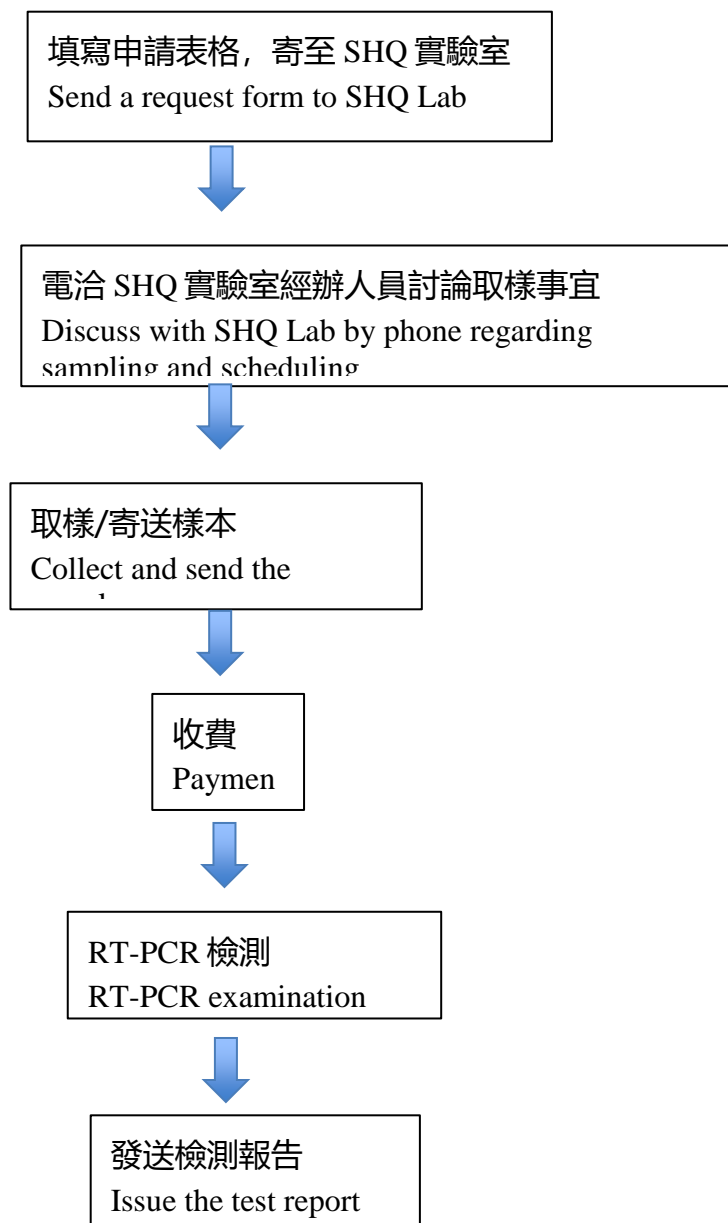
### Reference:

Lee, S.C., and Chang, Y.C. (2006). Multiplex RT-PCR detection of two orchid viruses with an internal control of plant *nad5* mRNA. Plant Pathol Bull 15, 187-196.

Verhoeven, J.t.j., Jansen, C.C.C., Willems, T.M., Kox, L.F.F., Owens, R.A., and Roenhorst, J.W. (2004). Natural infections of tomato by *Citrus exocortis viroid*, *Columnea latent viroid*, *Potato spindle tuber viroid* and *Tomato chlorotic dwarf viroid*. Eur J Plant Pathol 110, 823-831.  
<http://dx.doi.org/10.1007/s10658-004-2493-5>

圖一、檢測流程圖

Fig 1 Flow chart



圖二、樣品袋標示範例 Fig 2 Example of information needed on the sample bag





附件一、檢測申請表

Attachment: Request form

公司/機關名稱 Company/Institute Name	
申請日期 (年/月/日) Date (YYYY/MM/DD)	
收據抬頭 Title for the receipt	
統一編號 Company Tax ID	
聯絡人姓名 Contact	
地址 Address	
電話 Telephone	
電子郵件 E-mail	

匯款資訊 Wire transfer information :

行庫代號 Bank code : 700

郵局帳戶名稱 : 亞洲蔬菜研究發展中心

Account Name: Asian Vegetable Research and Development Center

存簿帳號 Account Number : 0191457-0002504

樣品資料 Sample information : (若不敷使用請自行加頁 Add pages if you need more space)

受理編號 SHQ No.	作物名稱 Crop	學名 Scientific name	品種名稱 Variety name	批號 Lot No.	樣品重量(公克) Sample weight (g)	備註 Remark