羅氏超視野組織免疫染色鑑定組

Roche *ultra*View™ Universal DAB Detection Kit

目錄號 760-500

限由醫師或醫檢師使用
適應症和用途
效能
此檢測套組是供體外診斷使用。
Ventana Medical Systems(Ventana)的超視野組織免疫染色鑑定組是用以偵測老鼠IgG、老鼠IgM和兔一級抗體的一個間接、不含生物素的系統。此套組在設計上是以免疫組織化學染色法(IHC)去確認經福馬林固定、石蠟包埋的組織切片及冰凍切片內的標的物(而這些切片是在Ventana NexES和BenchMark系列的自動切片染色機上被染色)。任何染色或沒有染色之臨床判讀是必需用形態學研究與適當對照的評估去互補之。必需要由一位合格的病理科醫師結合病患的病歷及其他診斷測試一起去進行評估。

總結和說明
免疫組織化學染色法(IHC)是在實驗室內使用而具有診斷目的的一種技術。IHC的基本概念是使用特異的一級抗體去辨認組織切片內抗原的位置。一旦發生抗原-抗體結合之後，必定可以藉由光學顯微鏡或螢光顯微鏡可見的一個呈色反應而觀察到這種抗原-抗體結合。特異的一級抗體可以帶有信號產生分子，從而對結合物提供了直接的觀察。另外，間接的觀察方法則需要額外步驟去確定特異性抗體的位置及產生一個信號。間接的方法中最常見的技術是使用一個對抗一級抗體的動物種之二級抗體及一個具有對應的基質呈色原系統的酵素。這個組合使得在特異性抗體的結合位置上形成了一個有顏色的沈澱物。超視野組織免疫染色鑑定組使用了一個間接方法，藉由一個棕色沈澱物的沈積而觀察到與抗原結合在一起的特異性抗體。

原理和過程
超視野組織免疫染色鑑定組是偵測與石蠟包埋或冰凍組織切片內的抗原結合在一起的特異性老鼠與兔一級抗體。使用一個酵素標記的二級抗體去確定此特異性抗體的位置。其後再利用一個可形成沈澱物的酵素產物去觀察這複合物。
超視野組織免疫染色鑑定組使用了以酵素標記的二級抗體(其可以對已結合的一級抗體進行定位)之一個混合物。其後再用基質過氧化氫和呈色原3, 3'–diaminobenzidine tetrahydrochloride (DAB)去觀察這複合物(過氧化氫和DAB可以形成一個容易地被光學顯微鏡偵測的深棕色沈澱物)。每個步驟均是在準確的時間和溫度下被孵育。在每個孵育步驟的結束時，Ventana自動切片染色機會清洗切片以除去未結合的物質，並且加上一層液體蓋玻片(可以使玻片上水溶性試劑的蒸發減到最少)。[1] 使用光學顯微鏡去判讀結果，而這些結果是可以輔助病理生理過程的鑒別診斷(這些病理生理過程是可以或可以不與一個特定的抗原有關連)。
有關儀器操作的詳細資訊，可參閱適當的Ventana自動切片染色機之操作手冊。

材料和方法
已提供的試劑
超視野組織免疫染色鑑定組含有足夠250個測試用的試劑，而此套組內含有:
1 – 25 ml的抑制劑分注器；內含3%過氧化氫溶液
1 – 25 ml的通用HRP多體(Universal HRP Multimer)分注器；在含有蛋白質的緩衝液中含有以HRP標記的抗體之混合物(山羊抗老鼠IgG抗體、山羊抗老鼠IgM抗體、及山羊抗兔抗體) (<50 µg/mL)，並加有ProClin 300保存劑*
1 – 25 ml的DAB呈色原分注器；在一個專利的穩定劑溶液中含有3, 3' – diaminobenzidine tetrahydrochloride，並加有專利的保存劑
1 – 25 ml的DAB H2O2分注器；在一個磷酸鹽緩衝溶液中含有0.04%過氧化氫
1 – 25 ml的銅分注器；在一個醋酸鹽緩衝液中含有硫酸銅(5g/l)，並加有專利的保存劑
*有關保存劑的資訊，請參考"預防注意事項"的部份。

重新泡製、混合、稀釋、滴定
此檢測套組在Ventana自動切片染色機上使用時已被最適化。套組試劑並不需要重新泡製、混合、稀釋、或滴定。額外的稀釋可以造成抗原染色的丟失。使用者必需查核是否有任何這樣的改變發生。在使用者的實驗室裡，組織處理與技術過程上的差異可以令結果產生很大的變化，因而需要習慣性地使用對照(參閱品管程序的部份)。

需要但未提供的材料和試劑
在染色時是需要以下未提供的試劑和材料:
1. 陰性試劑對照(Ventana陰性對照老鼠Ig (Cat No 760-2014), CONFIRM陰性對照兔Ig (Cat No 760-1029))
2. Superfrost Plus(VWR Cat. No. 48311-703或等同的)顯微鏡玻片
3. 陽性和陰性組織對照(有關建議的種類，參閱抗體的包裝內頁)
4. 能夠維持溫度60° C ± 5° C的烘箱
5. 條碼標籤(適用於陰性試劑對照和被測試的一級抗體)
6. 染色壺或染色槽
7. 計時器(有2-10分鐘的間隔)
8. 二甲苯(組織學級)
9. 乙醇或試劑酒精(組織學級)
   - 100%溶液：未稀釋的乙醇或試劑酒精
   - 95%溶液：將95份體積的乙醇或試劑酒精與5份體積的去離子水混合
   - 80%溶液：將80份體積的乙醇或試劑酒精與20份體積的去離子水混合
10. 去離子水或蒸餾水
11. Biocare Medical的免疫組織化學染色專用修護儀(Decloaking Chamber, Cat No DC2002) (NexES IHC自動切片染色機)*
12. Tissue-Tek染色皿*
13. NexES IHC、BenchMark系列的自動切片染色機
14. 一級抗體
15. Ventana抗體稀釋液* (Cat No 251-018)
16. Ventana APK清洗液 (10X) (Cat. No. 250-042) (NexES IHC自動切片染色機)
17. Ventana液體蓋玻片(低溫) (Cat. No. 250-009) (NexES IHC自動切片染色機)
18. Ventana EZ Prep (10X) (Cat. No. 950-102) (BenchMark系列的自動切片染色機)
19. Ventana反應緩衝液(10X) (Cat. No. 950-300) (BenchMark系列的自動切片染色機)
20. Ventana液體蓋玻片(高溫) (Cat. No. 650-010) (BenchMark系列的自動切片染色機)
21. Ventana細胞調節液1(預先稀釋) (Cat. No. 950-124)或細胞調節液2(預先稀釋) (Cat No 950-123) (BenchMark系列的自動切片染色機)*
22. Ventana蛋白酶1 (Cat No 760-2018)、蛋白酶2 (Cat No 760-2019)、或蛋白酶3(Cat No 760-2020)*
23. Ventana蘇木紫複染劑* (Cat No 760-2021)
24. Ventana蘇木紫II複染劑* (Cat. No. 790-2208)
25. Ventana調藍試劑* (Cat. No. 760-2037)

26. 永久封片膠(Permount, Fisher Cat. No. SP15-500或等同的)
27. 蓋玻片(足以覆蓋組織，例如VWR Cat. No. 48393-60或等同的)
28. 光學顯微鏡 (20-80X)
29. 吸水擦拭紙*
30. 自動封片機(例如Tissue-Tek SCA自動封片機)

*對於特定的應用是需要的。

貯存和處理

貯存於2 - 8° C。不要冰凍。除了那些在包裝內頁上指定的貯存條件外，其他任何的貯存條件都必需由使用者去確認。從冰箱裡取出此檢測套組之後可立即使用。

爲了確保適當的試劑傳送與每個試劑的穩定性，在每次運轉後必需蓋回蓋子及必需將分注器立即垂直地放回冰箱裡。

每套檢測套組都有有效日期。當試劑是適當地貯存時穩定性是可以直至標籤上所示的日期。不要使用超過了有效日期的檢測套組。

由於本產品的不穩定性並沒有決定性的徵象去顯示出來；所以，陽性和陰性對照必需與未知檢體同時地一起分析。如果顯示了試劑不穩定的現象，則必需立即聯絡你當地的Ventana公司。

爲分析而進行的檢體收集及處理

經福馬林固定、石蠟包埋的組織及冰凍組織都是適合與*超視野組織免疫染色鑑定組*和Ventana自動切片染色機一起使用(參閱需要但未提供的材料和試劑之部份)。

建議的組織固定液是10%中性緩衝福馬林溶液。[2] 由於長時間的固定或特殊過程(如骨髓組織製作時的去鈣化)，可出現不一定的結果。

每塊切片必需根據所用的一級抗體而被切到適合的厚度(2-5μ)，並放置在一片玻片上。含有組織切片的玻片可以放在60° C ± 5° C的烘箱內烘乾/加熱至少2小時(但不要超過24小時)。將玻片加熱是用以乾燥封片後的的組織及加強組織對玻片的黏著力。組織加熱時間過久會造成抗原表現性的減弱。參閱一級抗體的包裝內頁以確認加熱的限制。

適當地固定與包埋而可表現抗原的組織若貯存於陰涼的位置時(15° C - 25° C)，將可以保持最少2年的穩定性。在1988年的臨床檢驗室改進法案(Clinical Laboratory Improvement Act, CLIA)的42CFR493.1259 (b)裡要求"檢驗室必需從檢查當天起保留玻片最少十年及從檢查當天起保留標本塊最少兩年"。

冰凍切片必需根據所用的一級抗體而被切到適合的厚度(4-5μ)，並放置在一片顯微鏡玻片上及立即浸在冷丙酮(4° C - 8° C)中10分鐘。其後風乾切片最少三十分鐘(最好是整夜)。將適當的條碼標籤貼在已乾的玻片上。

手工脫蠟過程

當使用NexES IHC自動切片染色機或假若在BenchMark系列的自動切片染色機上沒有選擇脫蠟時均需要進行手工脫蠟:

1. 有關何時使用條碼標籤去標記玻片的說明指示，參閱特定的自動切片染色機操作手冊之使用說明部份。
2. 將玻片連續地浸在3個二甲苯染色槽中(在每個槽內浸5 ± 1分鐘)。
3. 將玻片轉移到100%乙醇，並將玻片連續地浸在2個染色槽中(在每個槽內浸3 ± 1分鐘)。
4. 將玻片轉移到95%乙醇，並將玻片浸在含有此溶液的染色槽中3 ± 1分鐘。
5. 將玻片轉移到80%乙醇，並將玻片浸在含有此溶液的染色槽中3 ± 1分鐘。
6. 將玻片轉移到含有去離子水或蒸餾水的染色槽中並浸洗最少10次。
7. 將玻片轉移到APK清洗液(1X)或適當的緩衝溶液。如果是用APK清洗液，則必需將玻片留置在此溶液中，直到你準備進行染色運轉。如果是用緩衝溶液，則必需將玻片留置在此溶液中，直到你準備進行抗原修復(antigen unmasking)過程。不要讓玻片乾掉。

在BenchMark系列的自動切片染色機上染色的玻片是可以在此儀器上進行脫蠟。如果選擇了此選項，將條碼標籤貼在玻片上並將玻片放在儀器上。如果沒有選擇此選項，則遵循以上的手工脫蠟過程。

預防注意事項

供體外診斷使用:

1. 當處理試劑時要採取適當的預防措施。當處理疑似致癌物質或毒性物質(例如: 二甲苯或甲醛)時要使用丟棄式手套。不要在開放的火焰(open flame)附近使用這些物質。
2. 避免試劑接觸到眼睛和黏膜。如果試劑接觸到敏感的部位，要用大量清水沖洗之。
3. 病患檢體及與病患檢體接觸的全部物質都必需被當作生物危險性物質去處理，並且採用適當的預防措施去丟棄之。千萬不可用口去吸取。
4. 試劑要避免受到微生物的污染，因爲這樣會產生不正確的結果。
5. 除了那些指定的孵育時間和溫度，其他的孵育時間和溫度可以產生錯誤的結果。使用者必需確認任何這樣的改變。
6. 試劑已經過最適當的稀釋，而額外再稀釋可以造成抗原染色的丟失。使用者必需確認任何這樣的改變。不要從本產品的多個批次去混合試劑分注器。
7. 過度暴露於ProClin 300的症狀是包括皮膚和眼睛的刺激、以及黏膜和上呼吸道的刺激。在本產品裡的ProClin 300濃度是少於或等於0.05%，因而並沒有達到美國職業安全衛生總署(OSHA)對於危險性物質的標準。敏感的個體可能出現全身性過敏反應。專利的保存劑和穩定劑並沒有達到美國職業安全衛生總署對於危險性物質的標準。
8. 可能的致癌物質。國家毒理學計劃(National Toxicology Program) 已經將聯苯胺 (benzidine)–一個與 3, 3' – diaminobenzidine tetrahydrochloride (DAB)非常類似的化合物 –列爲已知的人類致癌物質。
9. 濃縮型的丙二醇在實驗室動物中是已經與致畸胎作用有關連。當處理時要使用丟棄式手套及採取適當的預防措施。
10. 關於建議的丟棄方法，要向當地或國家的權責單位洽詢。

使用說明

一步一步的過程

Ventana *超視野組織免疫染色鑑定組*是爲了在Ventana自動切片染色機上使用(與Ventana一級抗體和附屬試劑合併使用)而被開發出來的。根據個別自動切片染色機的操作手冊內之過程，自動化過程的參數是可以被顯示、列印及編輯。自動切片染色機的其他操作參數在出廠前已被設定好。

在Ventana自動切片染色機上進行染色的過程是如以下所述。有關更詳細的說明和附加的方法(protocol)選項，可參閱你的操作手冊。一個檢體是否需要抗原修復是視抗體而定。請查閱抗體的包裝內頁以得到說明指示。

NexES IHC自動切片染色機

如果需要手工的抗原修復:

1. 經過一連串的二甲苯與梯度酒精、水、然後適當緩衝液的處理(如抗體包裝內頁所指示)，玻片得以被脫蠟。
   進行抗原修復過程(如抗體包裝內頁所指示)，以及將玻片轉移到APK 清洗液(1X)。
2. 將一級抗體、適當的檢測套組分注器及所需的附屬試劑裝載在試劑盤上，並將試劑盤放在自動切片染色機上。檢查大量使用的溶液(bulk fluids)和廢液。
3. 將玻片本身有顏色的一端乾燥，然後貼上玻片條碼標籤(此條碼標籤是與欲進行的抗體方法相對應的)。
4. 從 APK 清洗液(1X)中取出已脫蠟、抗原已修復、已貼標籤的玻片，並將它們裝載在 NexES IHC 自動切片染色機上。必需將玻片留置在此溶液中，直到準備進行染色運轉。

NexES IHC自動切片染色機及手工染色

如果不需要抗原修復:

1. 在玻片上貼上條碼標籤。經過一連串的二甲苯與梯度酒精、水、然後APK清洗液(1X)的處理，玻片得以被脫蠟。
2. 將一級抗體、適當的檢測套組分注器及所需的附屬試劑裝載在試劑盤上，並將試劑盤放在自動切片染色機上。檢查大量使用的溶液和廢液。
3. 從 APK 清洗液(1X)中取出已脫蠟及已貼標籤的玻片，並將它們裝載在 NexES IHC 自動切片染色機上。必需將玻片留置在此溶液中，直到準備進行染色運轉。

BenchMark系列的自動切片染色機

1. 貼上玻片條碼標籤(此條碼標籤是與欲進行的抗體方法相對應的)。
2. 將一級抗體、適當的檢測套組分注器及所需的附屬試劑裝載在試劑盤上，並將試劑盤放在自動切片染色機上。檢查大量使用的溶液和廢液。
3. 將玻片裝載在自動切片染色機上。

對於所有儀器

1. 開始染色運轉。
2. 在運轉完成的時候，從自動切片染色機上取出玻片。
3. 用一種含有溫和洗碗精或稀釋酒精的水溶液去清洗玻片，藉以除去蓋玻片溶液；並以慣常的方式去脫水及使用永久封片膠蓋上蓋玻片。
4. 從染色平台上取出玻片後可立即將其判讀。已染色的玻片如果適當地貯存在室溫(15° C - 25° C)下，則穩定性最少有兩年。

品管程序

陽性組織對照

在進行每個染色過程時必需同時執行一個陽性組織對照。陽性染色的組織成份是用來確認抗體已被加入及儀器是已正確地運作。這組織可以含有陽性和陰性的染色細胞或組織成份，而可以作爲陽性和陰性對照組織。

對照組織必需是儘快製作或固定的新鮮屍體解剖、活組織切片或外科手術檢體(使用與測試切片一樣的方法去製作或固定)。這些組織可以監控過程裡的所有步驟，包括由組織製作到染色。使用一個與測試檢體有不同固定或處理方法的組織切片，將會對全部試劑及方法步驟(除了固定和組織處理)提供了監控作用。

一個弱陽性染色的組織是更適合用於最理想的品質管控及偵測低水平的試劑降解。

已知的陽性組織對照必需只用於監控已處理的組織及檢測試劑是否有正確表現性，而不能作爲對病患檢體作出一個特定的診斷時的輔助。如果陽性組織對照無法顯現陽性的染色，則測試檢體的結果必需被考慮爲不正確。

陰性組織對照

用於陽性組織對照的同一個組織也可以當作陰性組織對照使用。存在於大部份組織切片裡的多個細胞種類提供了內部陰性對照的位置，但這必定要由使用者去確認。不染色的成份必需顯現爲沒有特異性染色及提供了一個背景染色的指標。如果陰性組織對照的位置出現特異性染色，則病患檢體的結果必需被考慮爲不正確。

無法解釋的不一致

如果對照出現了無法解釋的不一致，則必需立即聯絡你當地的Ventana公司。如果品管結果不符合標準規範，則病患的結果是不正確。如果出現了不一致的情形，可參閱本包裝內頁疑難排解的部份。找出問題並改正之，然後重覆病患檢體的分析。

陰性試劑對照

每個檢體都必需要執行一個陰性試劑對照，藉以輔助結果判讀。使用陰性試劑對照去代替一級抗體，從而評估非特異性染色。必需用適當的陰性對照老鼠Ig、CONFIRM陰性對照兔Ig、或兔陰性對照去將玻片染色。如果使用其他的陰性試劑對照，以Ventana抗體稀釋液將其稀釋至與一級抗體抗血清相同的濃度。而稀釋液也可以單獨地被用作先前提及的陰性試劑對照之另一個選擇。陰性試劑對照的孵育時間必需與一級抗體的孵育時間相同。

當含有數個抗體的套組是用於連續切片上的時候，在一片玻片上的一個陰性試劑對照可以作爲其他抗體的一個陰性或非特異性結合的背景對照。

分析確認

當一個一級抗體或染色系統被初次應用於一個診斷程序之前，必需在一系列具有已知的免疫組織化學表現特性的組織上進行測試，藉以確認一級抗體的特異性(具有已知的免疫組織化學表現特性的組織意指已知的陽性和陰性組織(可查閱一級抗體包裝內頁內所列的品管程序，以及美國病理學會實驗室認證計畫，解剖病理核對清單[3]、或CLSI認可的指引[4] 或這兩份文件))。對於每個新的抗體批次都必需重覆這些品管程序，或每當分析參數有更改時也要。

結果判讀

Ventana *超視野組織免疫染色鑑定組*使得一個深棕色的反應產物沈澱在由一級抗體所定位的抗原位置上。一位合格的、對免疫組織化學程序有經驗的病理科醫師在判讀結果之前必需評估對照及檢查染色產物的品質是否合格。首先必定要檢視陰性對照的染色，而這些陰性對照的染色結果要與染色物質作比較，藉以確認所產生的信號並不是來自非特異性的交互作用。

陽性組織對照

必需先檢視已染色的陽性組織對照，藉以確定全部的試劑都是正確地作用。在標的細胞內存在一個適當地染色的反應產物是表示陽性反應。視孵育時間的長短及所用的蘇木紫之效力而定，複染將會使細胞核產生一個淡藍至深藍的染色。過度或不完全的複染都可以影響結果判讀的正確性。

如果陽性組織對照無法顯現陽性的染色，則測試檢體的任何結果必需被考慮爲不正確。

陰性組織對照

在檢視陽性組織對照之後必需檢視陰性組織對照，藉以確認由一級抗體對標的抗原所產生的特異性標記。如果在陰性組織對照中沒有特異性染色，則確認了與細胞或細胞成份有交叉反應的抗體是並不存在。

如果陰性組織對照出現特異性染色，則病患檢體的結果必需被考慮爲不正確。

非特異性染色(如存在的話)將會有一個彌散的外觀。如果切片是來自經福馬林過度地固定的組織，也可以看到結締組織呈分散的淡染(sporadic light staining)。在判讀染色結果時必需用完整的細胞。壞死或變性的細胞常常被非特異地染色。

病患組織

病患檢體必需最後才被檢視。必需配合陰性試劑對照的任何非特異性背景染色之結果一起去評估陽性染色的強度。

如同任何的免疫組織化學測試，一個陰性的結果表示標的抗原沒有被偵測到，但並不是所分析的細胞或組織中沒有該抗原。如有需要，可使用一個抗體套組去輔助假陰性反應的確認。當判讀任何的免疫組織化學染色結果時，必需使用一片經蘇木紫-伊紅染色的切片去觀察每個組織樣本的形態。病患的形態學發現和有關的臨床數據必定要由一位合格的病理科醫師判讀。

限制

一般的限制

1. 免疫組織化學染色是一個多步驟的診斷過程，而對於適當試劑與組織的選擇、固定、處理、免疫組織化學玻片的製作、及染色結果的判讀均需要專業的訓練。
2. 組織染色是取決於染色前的組織處理和製作。不正確的固定、冷凍、解凍、清洗、乾燥、加熱、切片方法、或被其他組織或體液污染都可以產生人造物、抗體捕捉(antibody trapping)或假陰性的結果。不一致的結果可能是由於固定和包埋方法的差異、或組織內本身固有的不規則性所致。

3. 過度或不完全的複染都可以影響結果判讀的正確性。
4. 任何陽性染色(或沒有陽性染色)的臨床判讀必定要配合病歷、形態學和其他組織病理學標準一起評估。任何染色(或沒有染色)的臨床判讀必定要用形態學研究、適當的對照及其他診斷性測試去互補。在判讀染色的玻片時，一位合格的病理科醫師是有責任要熟悉所使用的抗體、試劑和方法。染色必定要由一位病理科醫師的監督下在一家已認證及有執照的實驗室內執行(此病理科醫師負責檢查染色的玻片及確保陽性和陰性對照的正確性)。
5. 當遵循內附的說明指示時，Ventana提供了具有最理想稀釋度的試劑以供使用。如果對建議的測試程序有任何違背，則可能使預期結果變得失效。必定要使用適當的對照及以書面型式記錄對照的染色結果。如果使用者違背了建議的測試程序，則必定要對病患結果的判讀承擔起責任。
6. 試劑可能在先前未測試過的組織中顯現非預期的反應。甚至在已測試過的組織群中出現非預期反應的可能性也是不能完全排除，因爲在腫瘤或其他病理組織的抗原表現是具有生物差異性。[5] 有關以書面記錄的非預期反應，可聯絡你當地的Ventana公司。
7. 如果組織是來自感染了B型肝炎病毒的個體並且含有B型肝炎表面抗原(HBsAg)，則與辣根過氧化酶反應後可能會顯現非特異性染色。[6]
8. 當使用在阻滯(blocking)步驟時，來自與二級抗血清相同的動物來源之正常血清可能會產生偽陰性或偽陽性結果，這是由於自體抗體或天然抗體所致。
9. 由於蛋白質或基質反應產物的非免疫性結合，可能會看到偽陽性結果。這些偽陽性結果也可能是由偽過氧化酶(pseudoperoxidase)活性(紅血球)及內生性過氧化酶活性(細胞色素C)所引起的。[7]
10. 如同任何的免疫組織化學測試，一個陰性的結果表示抗原沒有被偵測到，但並不是所分析的細胞或組織中沒有該抗原。

特別的限制

1. 檢測套組程序中的每個步驟都已在Ventana自動切片染色機上被最適化，並且都是先前已設定好的。由於組織固定和處理上的差異，個別檢體的一級抗體孵育時間可能需要增加或減少。有關固定時的變數(fixation variables)之進一步資訊，可參閱"免疫組織化學原理及進展"[8] 或"免疫鏡檢學：外科病理醫師的一個診斷工具"。[9]
2. 檢測套組與Ventana一級抗體和附屬試劑合併使用時，可偵測那些在經過常規福馬林固定、組織處理和切片後仍然存在的抗原。如果使用者違背了建議的測試程序，則要對病患結果的判讀和確認負起責任。

預期結果的總結

再現性研究

在BenchMark、BenchMark XT和NexES IHC自動切片染色機上，使用3種一級抗體對來自3個經中性緩衝福馬林固定並石蠟包埋的組織之連續切片進行染色(在乳癌組織上染色的老鼠IgG(抗Ki67)、在一個何杰金氏淋巴瘤異種移植體上染色的老鼠IgM(抗CD15)及在黑色素瘤組織上染色的兔IgG(抗S100))，從而進行*超視野組織免疫染色鑑定組*的再現性測試。全部一級抗體是被孵育16分鐘，並使用蘇木紫II接著調藍試劑去複染玻片。用同一種一級抗體染色的全部玻片是互相比較染色的適當性和強度，以及由一位合格的玻片判讀者評分。

1. 每天使用三種不同的染色機(BenchMark、BenchMark XT、和NexES IHC)各自執行一個染色運轉，共重覆三天(即總共執行了9個染色運轉)，從而計算運轉內再現性(在一個欲比較的平台上進行相同的一級抗體染色)。BenchMark XT的運轉內再現性是100%(每個運轉中每種一級抗體的30片玻片裡30片都有染色，而在全部3個運轉中對於每種抗體總共是染了90片玻片)，而BenchMark和NexES IHC的運轉內再現性也都是100%(每個運轉中每種一級抗體的20片玻片裡20片都有染色，而對於每個平台的全部3個運轉中每種抗體總共是染了60片玻片)。
2. 根據每種染色機的3個運轉內被染色的玻片數目去計算運轉間再現性。每天使用三種不同的染色機(BenchMark、BenchMark XT、和NexES IHC)各自執行一個染色運轉，共重覆三天。BenchMark XT的運轉間再現性是100%(90片玻片裡90片都有染色，在全部3個個別的運轉中對於每種一級抗體是染了30片玻片)，而BenchMark和NexES IHC的運轉間再現性也都是100%(60片玻片裡60片都有染色，對於每個平台的全部3個個別運轉中每種一級抗體是染了20片玻片)。
3. 根據全部3種染色機的9個運轉內被染色的玻片數目去計算儀器間再現性。每天使用三種不同的染色機(BenchMark、BenchMark XT、和NexES IHC)各自執行一個染色運轉，共重覆三天。*超視野組織免疫染色鑑定組*的儀器間再現性是100%(210片玻片裡210片都有染色，被評估的玻片包括全部3種一級抗體)。

另外，使用20種一級抗體對53個經中性緩衝福馬林固定的組織(包括正常及病態的)進行染色，從而測定*超視野組織免疫染色鑑定組*的表現性。所選擇的抗體都是代表了進行IHC時常用的動物種和類別。當由合格的玻片判讀者去判定時，*超視野組織免疫染色鑑定組*在100%受測試的個案(即53個染色的個案中的53個)都可以辨識到一級抗體並提供了適當的臨床應用性。

疑難排解

1. 如果陽性對照出現比預期較弱的染色，必需檢查同時執行的其他陽性對照，藉以判定原因是在一級抗體還是在其中一種常用的二級試劑。
2. 如果陽性對照是陰性，必需將其檢查，藉以確保玻片是有正確的條碼標籤。如果玻片上的條碼標籤是正確，則必需檢查同時執行的其他陽性對照，藉以判定原因是在一級抗體還是在其中一種常用的二級試劑。組織可能是已被不正確地採集、固定或脫蠟。對於採集、貯存和固定都必需遵循正確的程序。
3. 如果石蠟是未被全部除去，這樣可能會沒有染色。必需重覆脫蠟的過程。
4. 如果特異性抗體染色是太強，必需重覆染色運轉，而在重覆運轉時一級抗體的孵育時間是以4分鐘的時間間隔去縮短，直到需要的染色強度是被達到。
5. 如果組織切片從玻片上被沖洗掉，必需檢查玻片去確保它們是帶正電荷。
6. 有關修正措施，可參閱一步一步的過程之部份、自動切片染色機的操作手冊或聯絡你當地的Ventana公司。

參考文獻

1. Elias JM, Gown AM, Nakamura RM, Wilbur DC, Herman GE, Jaffe ES, Battifora H, Brigati DJ. Quality control in immunohistochemistry. Report of a workshop sponsored by the Biological Stain Commission. Am J Clin Pathol 92 (6): 836-843, 1989.
2. Sheehan DC, Hrapchak BB. Theory and practice of histotechnology, 2nd Edition. The C.V. Mosby Company, St. Louis, 1980.
3. College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist, 2001.
4. CLSI. Quality Assurance for Immunocytochemistry: Approved Guideline. CLSI document MM4-A- (ISBN 1-56238-396-5). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 1999.
5. Herman GE, Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech Histochem 66(4): 194-199, 1991.
6. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immunohistochemistry. Am J Clin Pathol 73(5): 626-32, 1980.
7. Nadji M, Morales AR. Immunoperoxidase: part 1. The technique and its pitfalls. Lab Med 14: 767, 1983.
8. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. (NR Rose Ed.) ASM Press, 2002.
9. Taylor C, Cote RJ. Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist, 2nd Edition. W.B. Saunders Company, Philadelphia. 1986.

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*超視野組織免疫染色鑑定組*是受正在申請中的專利保護。

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CE

*ultra*View™ Universal DAB Detection Kit

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This detection kit is intended for *in vitro* diagnostic use.

Ventana Medical Systems' (Ventana) *ultra*View Universal DAB Detection Kit is an indirect, biotin-free system for detecting mouse IgG, mouse IgM and rabbit primary antibodies. The kit is intended to identify targets by immunohistochemistry (IHC) in sections of formalin fixed, paraffin embedded and frozen tissue that are stained on the Ventana NexES® and BenchMark® Series automated slide stainers. The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

Summary and Explanation

Immunohistochemistry (IHC) is a technique used in laboratories for diagnostic purposes. The fundamental concept of IHC is the localization of antigens in tissue sections using specific primary antibodies. Once the antibody-antigen binding occurs it must be visualized with a colored reaction visible by light microscopy or through the use of fluorescence microscopy. The specific primary antibody may carry the signal generating molecule providing direct visualization of the binding. Alternatively, indirect methods of visualization require additional steps to localize the specific antibody and generate a signal. The most common techniques of indirect methods use a secondary antibody directed against the species of primary antibody and an enzyme with a corresponding substrate chromogen system. This combination results in a colored precipitate at the site of specific antibody binding. The *ultra*View Universal DAB Detection kit uses an indirect method to visualize specific antibodies bound to antigens by depositing a brown colored precipitate.

Principles and Procedures

The *ultra*View Universal DAB Detection Kit detects specific mouse and rabbit primary antibodies bound to an antigen in paraffin embedded or frozen tissue sections. The specific antibody is located by an enzyme labeled secondary antibody. The complex is then visualized utilizing a precipitating enzyme product.

The *ultra*View Universal DAB Detection Kit utilizes a cocktail of enzyme labeled secondary antibodies that locate the bound primary antibody. The complex is then visualized with hydrogen peroxide substrate and 3, 3' – diaminobenzidine tetrahydrochloride (DAB) chromogen, which produce a dark brown precipitate that is readily detected by light microscopy. Each step is incubated for a precise time and temperature. At the end of each incubation step, the Ventana automated slide stainer washes the sections to remove unbound material and applies a liquid coverslip which minimizes the evaporation of the aqueous reagents from the slide.[1] Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

For more detailed information on instrument operation, refer to the appropriate Ventana automated slide stainer Operator's Manual.

MATERIALS AND METHODS

Reagents Provided

*ultra*View Universal DAB Detection Kit contains sufficient reagent for 250 tests and contains:

1 – 25 ml dispenser Inhibitor; contains 3% hydrogen peroxide solution

1 – 25 ml dispenser Universal HRP Multimer; contains a cocktail of HRP labeled antibodies (goat anti-mouse IgG, goat anti-mouse IgM, and goat anti-rabbit) (<50 µg/mL) in a buffer containing protein with ProClin® 300 preservative*

1 – 25 ml dispenser DAB Chromogen; contains 3, 3' – diaminobenzidine tetrahydrochloride in a proprietary stabilizer solution with a proprietary preservative

1 – 25 ml dispenser DAB H_2O_2; contains 0.04% hydrogen peroxide in a phosphate buffer solution

1 – 25 ml dispenser Copper; contains copper sulfate (5g/l) in an acetate buffer with proprietary preservative

*For preservative information please refer to the "Warnings and Precautions" section.

Reconstitution, Mixing, Dilution, Titration

The detection kit is optimized for use on a Ventana automated slide stainer. No reconstitution, mixing, dilution, or titration of kit reagents is required.

Further dilution may result in loss of antigen staining. The user must verify any such changes. Differences in tissue processing and technical procedure in the user's laboratory may produce significant variability in results and require regular use of controls (see Quality Control Procedures section).

Materials and Reagents Needed But Not Provided

The following reagents and materials may be required for staining but are not provided:

1. Negative reagent control (Ventana Negative Control Mouse Ig (Cat No 760-2014), CONFIRM™ Negative Control Rabbit Ig (Cat No 760-1029)
2. Superfrost™ Plus (VWR Cat. No. 48311-703 or equivalent) microscope slides
3. Positive and negative tissue controls (consult antibody package insert for recommended types)
4. Drying oven capable of maintaining a temperature of 60° C ± 5° C
5. Bar code labels (appropriate for negative reagent control and primary antibody being tested)
6. Staining jars or baths
7. Timer (capable of 2-10 minute intervals)
8. Xylene (Histological grade)
9. Ethanol or reagent alcohol (Histological grade)
   -100% solution: undiluted ethanol or reagent alcohol
   -95% solution: mix 95 parts ethanol or reagent alcohol with 5 parts of deionized water
   -80% solution: mix 80 parts ethanol or reagent alcohol with 20 parts deionized water
10. Deionized or distilled water
11. Biocare Medical's Decloaking Chamber (Cat No DC2002) (NexES IHC automated slide stainers)*
12. Tissue-Tek® staining dishes*
13. NexES IHC, BenchMark Series automated slide stainers
14. Primary antibody
15. Ventana Antibody Diluent* (Cat No 251-018)
16. Ventana APK Wash (10X) (Cat. No. 250-042) (NexES IHC automated slide stainers)
17. Ventana Liquid Coverslip™ (Low Temperature) (Cat. No. 250-009) (NexES IHC automated slide stainers)
18. Ventana EZ Prep™ (10X) (Cat. No. 950-102) (BenchMark Series automated slide stainers)
19. Ventana Reaction Buffer (10X) (Cat. No. 950-300) (BenchMark Series automated slide stainers)
20. Ventana Liquid Coverslip (High Temperature) (Cat. No. 650-010) (BenchMark Series automated slide stainers)
21. Ventana Cell Conditioning 1 (Pre-dilute) (Cat. No. 950-124) or Cell Conditioning 2 (Pre-dilute) (Cat No 950-123) (BenchMark Series automated slide stainers)*
22. Ventana Protease 1 (Cat No 760-2018),Protease 2 (Cat No 760-2019), or Protease 3 (Cat No 760-2020)*
23. Ventana Hematoxylin counterstain* (Cat No 760-2021)
24. Ventana Hematoxylin II counterstain* (Cat. No. 790-2208)
25. Ventana Bluing Reagent* (Cat. No. 760-2037)
26. Permanent Mounting Medium (Permount®, Fisher Cat. No. SP15-500 or equivalent)
27. Cover glass (sufficient to cover tissue such as VWR Cat. No. 48393-60 or equivalent)
28. Light microscope (20-80X)
29. Absorbent wipes*
30. Automated coverslipper (such as Tissue-Tek® SCA automated coverslipper)
    *As needed for specific applications.

Storage and Handling

Store at 2° C to 8° C. Do not freeze. The user must validate any storage conditions other than those specified in the package insert. This detection kit can be used immediately after removal from the refrigerator.

To ensure proper reagent delivery and stability of each reagent, after every run the cap must be replaced and the dispenser must be immediately placed in the refrigerator in an upright position.

Every detection kit is expiration dated. When properly stored, the reagents are stable to the date indicated on the label. Do not use detection kit beyond the expiration date.

There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens. Your local Ventana office should be contacted immediately if there is an indication of reagent instability.

Specimen Collection and Preparation for Analysis

Formalin fixed, paraffin embedded tissues and frozen tissues are suitable for use with *ultra*View Universal DAB Detection Kit and a Ventana automated slide stainer (see Materials and Reagents Needed, But Not Provided section). The recommended tissue fixative is 10% neutral buffered formalin.[2] Variable results may occur as a result of prolonged fixation or special processes such as decalcification of bone marrow preparations.

Each section should be cut to the appropriate thickness (2-5µ) for the primary antibody being used and placed on a glass slide. Slides containing the tissue section may be baked/heated for at least 2 hours (but not longer than 24 hours) in a 60° C ± 5° C oven. Slide heating is used to dry the tissue post slide mounting and to enhance tissue adhesion to the glass. Extended heating of the tissue might result in decreased antigen availability. Consult the primary antibody package insert to identify heating limitations.

Properly fixed and embedded tissues expressing the antigen will remain stable for at least 2 years if stored in a cool location (15° C - 25° C). The Clinical Laboratory Improvement Act (CLIA) of 1988, 42CFR493.1259 (b) requires that "The laboratory must retain slides at least

ten years from the date of examination and retain specimen blocks at least two years from date of examination".

Frozen sections should be cut to the appropriate thickness (4-5μ) for the primary antibody being used, picked up on a glass microscope slide and immediately placed in cold acetone (4° C to 8° C) for ten minutes. The sections are then air dried for a minimum of thirty minutes and preferably overnight. Apply appropriate barcode label to dry slides.

Manual Deparaffinization Procedure

Required when using the NexES IHC automated slide stainer or if deparaffinization is not selected on the BenchMark Series automated slide stainer:

1. For instructions on when to label slides with barcode label, refer to the Instructions for Use section of the specific automated slide stainer Operator's Manual.
2. Immerse the slides sequentially in 3 xylene baths for 5 ± 1 minutes each.
3. Transfer the slides to 100% ethanol and immerse sequentially in 2 baths for 3 ± 1 minutes each.
4. Transfer the slides to 95% ethanol and immerse them in a bath of this solution for 3 ± 1 minutes.
5. Transfer the slides to 80% ethanol and immerse them in this solution for 3 ± 1 minutes.
6. Transfer the slides to a bath of deionized or distilled water and dip a minimum of 10 times.
7. Transfer slides to APK Wash (1X) or buffer solution as appropriate. For APK Wash, the slides should remain in this solution until you are ready to perform the staining run. For buffer solution, the slides should remain in this solution until you are ready to perform the antigen unmasking procedure. Do not allow the slides to dry.

Slides stained on the BenchMark Series automated slide stainers can be deparaffinized on the instrument. If this option is selected, apply barcode labels to slides and place slides on the instrument. If the option is not selected, follow the Manual Deparaffinization Procedure above.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use:

1. Take reasonable precautions when handling reagents. Use disposable gloves when handling suspected carcinogens or toxic materials (example: xylene or formaldehyde). Do not use near open flame.
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
3. Patient specimens and all materials contacting them should be handled as biohazardous materials and disposed of with proper precautions. Never pipette by mouth.
4. Avoid microbial contamination of reagents, as this could produce incorrect results.
5. Incubation times and temperatures other than those specified may give erroneous results. The user must validate any such change.
6. The reagents have been optimally diluted, and further dilution may result in loss of antigen staining. The user must validate any such change. Do not mix reagent dispensers from multiple lots of the product.
7. Symptoms of overexposure to ProClin® 300 include skin and eye irritation, and irritation of mucous membranes and upper respiratory tract. The concentration of ProClin® 300 in this product is less than or equal to 0.05% and does not meet the OSHA criteria for a hazardous substance. Systemic allergic reactions are possible in sensitive individuals. Proprietary preservatives and stabilizers do not meet the OSHA criteria for a hazardous substance.
8. Possible carcinogen. The National Toxicology Program has listed benzidine, a closely related compound to 3, 3' – diaminobenzidine tetrahydrochloride (DAB), as a known human carcinogen.
9. Concentrated forms of propylene glycol have been associated with teratogenic effects in laboratory animals. Use disposable gloves and take reasonable precautions when handling.
10. Consult local or state authorities with regard to recommended method of disposal.

INSTRUCTIONS FOR USE

Step by Step Procedure

Ventana *ultra*View Universal DAB Detection Kit has been developed for use on Ventana automated slide stainers in combination with Ventana primary antibodies and accessories. The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the Operator's Manual for the individual automated slide stainer. Other operating parameters for the automated slide stainers have been preset at the factory.

The procedures for staining on the Ventana automated slide stainers are as follows. For more detailed instructions and additional protocol options refer to your Operator's Manual. Whether a sample requires Antigen Unmasking is antibody dependent. Please check the antibody package insert for directions.

NexES IHC Automated Slide Stainers
If Manual Antigen Unmasking Is Required:

1. Slides are to be deparaffinized through a series of xylene and gradient alcohols to water and then to appropriate buffer (as indicated by the antibody package insert). Perform antigen unmasking procedure (as indicated by the antibody package insert) and transfer slides to APK Wash (1X).
2. Load the primary antibody, appropriate detection kit dispensers and required accessory reagents onto the reagent tray and place the reagent tray on the automated slide stainer. Check bulk fluids and waste.
3. Dry the painted end of the slide and then apply slide barcode label that corresponds to the antibody protocol to be performed.
4. Load the deparaffinized, antigen unmasked, labeled slides from the APK Wash (1X) onto the NexES IHC Automated Slide Stainer. Slides should remain in this solution until ready to perform staining run.

NexES IHC Automated Slide Stainers and Manual
If Antigen Unmasking Is Not Required:

1. Apply barcode labels to the slide. Slides are to be deparaffinized through a series of xylene and gradient alcohols to water and then to APK Wash (1X).
2. Load the primary antibody, appropriate detection kit dispensers and required accessory reagents onto the reagent tray and place the reagent tray on the automated slide stainer. Check bulk fluids and waste.
3. Load the deparaffinized, labeled slides from the APK Wash (1X) onto the NexES IHC Automated Slide Stainer. Slides should remain in this solution until ready to perform staining run.

BenchMark Series Automated Slide Stainers

1. Apply slide barcode label that corresponds to the primary antibody protocol to be performed.
2. Load the primary antibody, appropriate detection kit dispensers and required accessory reagents onto the reagent tray and place the reagent tray on the automated slide stainer. Check bulk fluids and waste.
3. Load the slides onto the automated slide stainer.

For All Instruments

1. Start the staining run.
2. At the completion of the run, remove the slides from the automated slide stainer.
3. Wash slides in an aqueous solution containing a mild dishwashing detergent or dilute alcohol to remove the coverslip solution; dehydrate and coverslip with permanent mounting media in the usual manner.
4. Slides may be read immediately upon removal from the staining platform. Stained slides are stable for at least two years, if properly stored at room temperature (15° C to 25° C).

Quality Control Procedures

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. The positive staining tissue components are used to confirm that the antibody was applied and the instrument functioned properly. This tissue may contain both positive and negative staining cells or tissue components and may serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections. Such tissues may monitor all steps of the procedure, from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen will provide control for all reagents and method steps except fixation and tissue processing.

A tissue with weak positive staining is more suitable for optimal quality control and for detecting minor levels of reagent degradation.

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control

The same tissue used for the positive tissue control may be used as the negative tissue control. The variety of cell types present in most tissue sections offers internal negative control sites, but this must be verified by the user. The components that do not stain should demonstrate the absence of specific staining, and provide an indication of background staining. If specific staining occurs in the negative tissue control sites, results with the patient specimens should be considered invalid.

Unexplained Discrepancies

Unexplained discrepancies in controls should be referred to your local Ventana office immediately. If quality control results do not meet specifications, patient results are invalid. If discrepancies occur, refer to the Troubleshooting section of this insert. Identify and correct the problem, then repeat the patient samples.

Negative Reagent Control

A negative reagent control must be run for every specimen to aid in the interpretation of results. A negative reagent control is used in place of the primary antibody to evaluate nonspecific staining. The slide should be stained with Negative Control Mouse Ig, CONFIRM Negative Control Rabbit Ig, or Rabbit Negative Control as appropriate. If an alternative negative reagent control is used, dilute to the same concentration as the primary

antibody antiserum with Ventana Antibody Diluent. The diluent alone may be used as an alternative to the previously described negative reagent controls. The incubation period for the negative reagent control should equal the primary antibody incubation period.
When panels of several antibodies are used on serial sections, a negative reagent control on one slide may serve as a negative or nonspecific binding background control for other antibodies.

Assay Verification
Prior to initial use of a primary antibody or staining system in a diagnostic procedure, the specificity of the primary antibody should be verified by testing on a series of tissues with known immunohistochemistry performance characteristics representing known positive and negative tissues (refer to the Quality Control Procedures listed in the primary antibody product insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist[3], or the CLSI Approved Guideline[4] or both documents). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters.

Interpretation of Results

The Ventana *ultra*View Universal DAB Detection Kit causes a dark brown colored reaction product to precipitate at the antigen sites localized by the primary antibody. A qualified pathologist experienced in immunohistochemical procedures must evaluate controls and qualify the stained product before interpreting results. Staining of negative controls must be noted first, and these results compared to the stained material to verify that the signal generated is not the cause of nonspecific interactions.

Positive Tissue Control
The stained positive tissue control should be examined first to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product within the target cells is indicative of positive reactivity. Depending on the incubation length and potency of the hematoxylin used, counterstaining will result in a pale to dark blue coloration of cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results.
If the positive tissue control fails to demonstrate positive staining, any results with the test specimens should be considered invalid.

Negative Tissue Control
The negative tissue control should be examined after the positive tissue control to verify the specific labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross reactivity to cells or cellular components. If specific staining occurs in the negative tissue control, results with the patient specimen should be considered invalid.
Nonspecific staining, if present, will have a diffuse appearance. Sporadic light staining of connective tissue may also be observed in sections from excessively formalin fixed tissues. Intact cells should be used for interpretation of staining results. Necrotic or degenerated cells often stain nonspecifically.

Patient Tissue
Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any non-specific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen in question was not detected, not that the antigen is absent in the cells or tissue assayed. If necessary, use a panel of antibodies to aid in the identification of false negative reactions. The morphology of each tissue sample should also be examined utilizing a hematoxylin and eosin stained section when interpreting any immunohistochemical result. The patient's morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

LIMITATIONS

General Limitations

1. Immunohistochemistry is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents and tissues, fixation, processing, preparation of the immunohistochemistry slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be a consequence of variations in fixation and embedding methods, or inherent irregularities within the tissue.
3. Excessive or incomplete counterstaining may compromise proper interpretation of results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and proper controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents and methods used to interpret the stained preparation. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
5. Ventana provides reagents at optimal dilution for use when the provided instructions are followed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results.
6. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues.[5] Contact your local Ventana office with documented unexpected reactions.
7. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[6]
8. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results due to autoantibodies or natural antibodies.
9. False positive results may be seen because of nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C).[7]
10. As with any immunohistochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.

Specific Limitations

1. Each step of the detection kit procedure has been optimized on the Ventana automated slide stainers and is preset. Because of variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation time on individual specimens. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances"[8] or "Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist".[9]
2. The detection kit, in combination with Ventana primary antibodies and accessories, detects antigen that survives routine formalin, tissue processing and sectioning. Users who deviate from recommended test procedures are responsible for interpretation and validation of patient results.

SUMMARY OF EXPECTED RESULTS

Reproducibility Studies

*ultra*View Universal DAB Detection Kit reproducibility testing was performed by staining serial sections from 3 neutral buffered formalin fixed, paraffin embedded tissues using 3 primary antibodies, a mouse IgG (anti-Ki67) stained on breast carcinoma, a mouse IgM (anti-CD15) stained on a Hodgkin's Lymphoma xenograft and a rabbit IgG (anti-S100) on a melanoma using BenchMark, BenchMark XT and NexES IHC automated slide stainers. All primary antibodies were incubated for 16 minutes and slides counterstained using Hematoxylin II followed by Bluing Reagent. All slides stained with a primary antibody were compared against each other for staining appropriateness and intensity and scored by a qualified slide reader.

1. Intra run reproducibility (same primary antibody stained on a platform compared) staining runs were performed one per day on 3 separate days using 3 different stainers: BenchMark, BenchMark XT, & NexES IHC for a total of 9 staining runs. Intra run reproducibility for BenchMark XT was 100% (30 of 30 slides per primary antibody per run, a total of 90 slides stained per antibody across the 3 runs) and 100% (20 of 20 slides per primary antibody per run, a total of 60 slides per antibody across 3 runs for each platform: BenchMark and NexES IHC).
2. Inter run reproducibility was calculated based on the number of slides stained in 3 runs per stainer type. Staining runs were performed one per day on 3 separate days using 3 different stainers: BenchMark, BenchMark XT, & NexES IHC. Inter run reproducibility for BenchMark XT was 100% (90 of 90 slides, 30 slides for each primary antibody across 3 separate runs) and 100% (60 of 60 slides, 20 slides for each primary antibody across 3 separate runs for each platform) for BenchMark and NexES IHC.
3. Inter instrument reproducibility was calculated based on the number of slides stained in 9 runs across all stainer types. Staining runs were performed 1 per day on 3 separate days using 3 different stainers: BenchMark, BenchMark XT, & NexES IHC. *ultra*View Universal DAB Detection Kit inter instrument reproducibility is 100% (210 of 210 stained slides, evaluated slides included all 3 primary antibodies).

The performance of the *ultra*View Universal DAB Detection Kit was also determined by staining 53 normal and disease state neutral buffer formalin fixed tissues using 20 primary antibodies. The selected antibodies represented species and isotypes commonly used to perform IHC. In 100% of the cases tested (53 of 53 cases stained) *ultra*View Universal DAB Detection Kit recognized the primary antibody and provided the appropriate clinical utility as determined by the qualified slide reader.

TROUBLESHOOTING

1. If the positive control exhibits weaker staining than expected, other positive controls run concurrently should be checked to determine if it is due to the primary antibody or one of the common secondary reagents.

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2. If the positive control is negative, it should be checked to ensure that the slide has the proper barcode label. If the slide is labeled properly, other positive controls run concurrently should be checked to determine if it is due to the primary antibody or one of the common secondary reagents. Tissues may have been improperly collected, fixed or deparaffinized. The proper procedure should be followed for collection, storage and fixation.
3. If all of the paraffin has not been removed, there may be no staining. The deparaffinization procedure should be repeated.
4. If specific antibody staining is too intense, the run should be repeated with the primary antibody incubation time shortened by 4 minute intervals until the desired stain intensity is achieved.
5. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged.
6. For corrective action, refer to the Step By Step Procedure section, the automated slide stainer Operator's Manual or contact your local Ventana office.

REFERENCES

1. Elias JM, Gown AM, Nakamura RM, Wilbur DC, Herman GE, Jaffe ES, Battifora H, Brigati DJ. Quality control in immunohistochemistry. Report of a workshop sponsored by the Biological Stain Commission. Am J Clin Pathol 92 (6): 836-843, 1989.
2. Sheehan DC, Hrapchak BB. Theory and practice of histotechnology, 2nd Edition. The C.V. Mosby Company, St. Louis, 1980.
3. College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist, 2001.
4. CLSI. Quality Assurance for Immunocytochemistry: Approved Guideline. CLSI document MM4-A- (ISBN 1-56238-396-5). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 1999.
5. Herman GE, Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech Histochem 66(4): 194-199, 1991.
6. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. A possible source of error in immunohistochemistry. Am J Clin Pathol 73(5): 626-32, 1980.
7. Nadji M, Morales AR. Immunoperoxidase: part 1. The technique and its pitfalls. Lab Med 14: 767, 1983.
8. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. (NR Rose Ed.) ASM Press, 2002.
9. Taylor C, Cote RJ. Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist, 2nd Edition. W.B. Saunders Company, Philadelphia. 1986.

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*ultra*View Universal DAB Detection Kit covered by patents pending.

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