3. PPE 装着

場所：ナースステーションおよび廊下（本来なら前室であるが、訓練の便宜上ここを使用する）。各班 6 名前後

時間の目安：20 分

準備品：手袋（内側用、外側用）、ボディスーツ型ガウン、N95 マスク、ゴーグル、シューカバー

※どの PPE を選択するかは、各種の国際指針で一定しているわけではなく、欧州と米国では明らかな相違がある。別紙「ウイルス性出血熱患者を診療・ケアする際の PPE 選択」参照

※PPE の脱ぎ方は「4. マネキン訓練」の最後に行う

※

① 実習の便宜上、普通服の上に PPE を着用する
② 国立国際医療研究センター「図 4　フル PPE の着け方」に準じる
　ただし、耐水ディスポガウン、フェイスシールド、ブーツは省略する
③ まず、手袋（内側）を着ける
④ 以降「図 4　フル PPE の着け方」の通り
　ただし：手袋（内側）のすそはガウン袖に覆われる
　　手袋（外側）のすそはガウン袖を覆う
⑤ 班全員の装着を確認（**指導員チェック**）
⑥ 引き続き「4. マネキン訓練」に進む

4. **マネキン訓練**

場所：隔離病室内。各班 6 名前後

時間の目安：1 時間

準備品：ベッド、実習用マネキン、病衣、血圧計、生食＋輸液ライン、静脈留置針、擬似皮膚パッド、鋭利物廃棄容器、擬似吐物、吐物処理キット、廃棄物袋、救急カート（またはワゴン）、UV ランプ

※

※参加者すべてが順番に作業できるように、適宜声をかけてゆく

A. 初期対応（15 分）

① バイタルサイン測定、ナースステーションに報告

② ルート確保、針の確実な処理（**指導員チェック**）

B. 吐物対応（15 分）

① 指導員は、床に擬似吐物を撒く

② 吐物処理キットを取り出す

③ 一連の吐物処理（**指導員チェック**）

④ 体位交換しながら病衣を取り替える（**指導員チェック**）

C. 急変対応（10 分）

①

②

③

④

⑤

⑥

D. （20 分）

①

②

③

④ 汚染を残さないように注意しながら、PPE を外して廃棄物袋に入れる

国立国際医療研究センター「図 5　フル PPE の脱ぎ方」に準じる

ただし：

1. フェイスシールド、黄色のディスポガウン、ブーツは省略する
2. 「前室で空気の入れ替わりを待つ」も省略する
3. 手袋（内側）を外した時点で、汚染が残っていないかどうか確認（**指導員チェック**）
4. 最後に手洗い

ウイルス性出血熱患者に由来する血液・体液・吐物・下痢の消毒法

文献	作成者	対象国・地域	発表年	薬剤	反応時間
1	ACDP	英国	1996	ジクロロイソシアヌル酸顆粒	2 分
				1%次亜塩素酸	2 分
2	CDC/WHO	アフリカ諸国	1998	0.5%塩素系溶液	15 分
3	ENIVD	欧州諸国	2001	1%次亜塩素酸	30 分
4	CDC	米国	2005	0.5%次亜塩素酸	指定なし
5	ACDP	英国	2011	1%次亜塩素酸	2 分
6	消毒と滅菌のガイドライン	日本	2011	0.5%次亜塩素酸	拭き取り
				ジクロロイソシアヌル酸 Na 顆粒	5 分以上
参考	東京都ノロウイルス対策標準マニュアル	日本	2006	0.1%次亜塩素酸	拭き取り

文献

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2. Centers for Disease Control and Prevention and World Health Organization (1998) Infection Control for viral haemorrhagic fevers in the African health care setting.
3. European Network for Diagnostics of Imported Viral Diseases (2001) Management and control of viral haemorrhagic fevers. 2nd version
4. Centers for Disease Control and Prevention (2005) Interim guidance for managing patients with suspected viral hemorrhagic fever in U. S. hospitals.
5. Advisory Committee on Dangerous Pathogens (2011) Management of Hazard Group 4 viral haemorrhagic fevers and similar human infectious diseases of high consequence – technical stakeholder engagement exercise.
6. 小林編（2011）［新版］消毒と滅菌のガイドライン．へるす出版

ウイルス性出血熱患者を診療・看護する際の PPE 選択

文献	作成者	対象国・地域	発表年	ガウン	手袋	マスク	ゴーグル	エプロン	キャップ	靴カバー	備考
1	CDC	米国	1988	√	√	√	*			√	
2	CDC	米国	1995	√	√	サージカル†	√			‡	呼吸器症状がある場合、HEPA レスピレータ
3	WHO/CDC	アフリカ諸国	1998	√	二重	HEPA or サージカル or 木綿マスク	√	√	√	ゴム長靴	
4	ENIVD	欧州諸国	2001	耐水性	二重	陽圧 HEPA	√			√	アイソレータ使用
5	オンタリオ州	カナダ	2002	√	√	サージカル†	√			§	著明な咳や激しい下痢があれば、HEPA レスピレータ
6	CDC	米国	2005	√	√	サージカル†	√	‡		‡	肺病変があるか、エアロゾルが発生する操作を行う際は、airborne precaution
7	ACDP	英国	2011	耐水性	二重	FFP3 レスピレータ	√				バイザー
参考	国立国際医療研究センター	日本		√	二重	N95	√	√		√	フェイスシールド、長靴

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* 非協力的な患者や、嘔吐・出血を伴う操作の際

† サージカルマスク+ゴーグルに替えて、フェイスシールドでもよい

‡ 大量の血液・体液・吐物・便による汚染がある場合

§ 血液・体液に触れる操作を行う場合

文献

1. Centers for Disease Control and Prevention (1988) Management of patients with suspected viral hemorrhagic fever. MMWR 37(S-3);1-16
2. Centers for Disease Control and Prevention (1995) Notice to readers update: management of patients with suspected viral hemorrhagic fever – United States. MMWR 44(25);475-9
3. Centers for Disease Control and Prevention and World Health Organization (1998) Infection Control for viral haemorrhagic fevers in the African health care setting.
4. European Network for Diagnostics of Imported Viral Diseases (2001) Management and control of viral haemorrhagic fevers. 2nd version

5. Contingency Plan – Ontario (2002) Viral hemorrhagic fevers.
6. Centers for Disease Control and Prevention (2005) Interim guidance for managing patients with suspected viral hemorrhagic fever in U. S. hospitals.
7. Advisory Committee on Dangerous Pathogens (2011) Management of Hazard Group 4 viral haemorrhagic fevers and similar human infectious diseases of high consequence – technical stakeholder engagement exercise.

一類感染症ワークショップ
プレテスト・ポストテスト結果

	プレテスト	ポストテスト	前後の比較 (ペア t 検定)
医師	9.6 ± 1.4	10.9 ± 1.0	$p = 0.01$
看護師	6.9 ± 1.9	9.9 ± 1.0	$p = 0.00002$
全体	7.9 ± 2.2	10.3 ± 1.1	$p = 0.000002$
(満点)	12	12	

平均±標準偏差

＊　＊　＊

プレテスト（ポストテストも同一問題）

1. ラッサ熱が常在する地域は、次のうちどれか?

   a. トルコ・中央アジア
   b. 東アフリカ
   c. 西アフリカ
   d. 南米アマゾン川流域

2. ウイルス性出血熱の主症状は下血である。

   a. はい
   b. いいえ

3. 次のうち一類感染症に分類される疾患は?

   a. オムスク出血熱
   b. エボラ出血熱
   c. デング出血熱
   d. 腎症候性出血熱
   e. 上のすべて

4. ラッサ熱の鑑別診断であげられる疾患は次のどれか?

   a. マラリア
   b. デング熱
   c. 黄熱
   d. 腸チフス
   e. 上のすべて

5. 患者の臨床検体を搬送する場合に必要な梱包様式は?

a. 基本一重梱包
b. 基本二重梱包
c. 基本三重梱包

6. ラッサウイルスの取扱いには、次のいずれのバイオセーフティレベルが必要か?

a. BSL 1
b. BSL 2
c. BSL 3
d. BSL 4

7. ヒトからヒトへの直接感染が**想定されていないもの**は、次のうちどれか?

a. ラッサ熱
b. エボラ出血熱
c. マールブルク病
d. デング出血熱

8. ウイルス性出血熱の感染リスクが最も高いと考えられるのは、次のうちどれか?

a. 患者と同じ飛行機に、10 時間搭乗した
b. 患者の嘔吐物が素手に触れた
c. 個人用防護具を装着して、患者のバイタルサイン測定を行った
d. 患者と同じ部屋に、1 時間一緒にいた

9. ウイルス性出血熱の接触者調査について、次のうち正しいものはどれか?

a. 患者と接触した記憶がはっきりしない場合、感染リスクが高いと考えて扱う
b. 接触者の感染リスクが低い場合、接触機会から 7 日後まで経過を追跡する
c. 接触者の感染リスクが高い場合、接触機会から 21 日後まで毎日体温を記録すべきである
d. 接触者の感染リスクが高い場合、症状がなくても入院するのが望ましい

10. 呼吸器に病原性を持つ粒子のうち、最も小さいものはどれか?

a. ウイルス
b. 一般細菌
c. 結核菌
d. 花粉

11. 院内感染防止の視点から、最も有効な呼吸器防護対策と考えられるのはどれか?

a. 咳エチケットを普及させる
b. 適切な呼吸防護具を装着する
c. 感染者を早期に特定して誘導する
d. HEPA フィルターを設置する

12. 呼吸用保護具について、正しい文章はどれか?

a. N95 マスクは、最高の粒子捕集性能を持った呼吸用保護具である
b. 呼吸用保護具には、品質保証のための国家検定規格がある
c. サッカリンなどの味覚粒子を用いて漏れ率を測定する方法は、定量的フィットテストと呼ばれる
d. フィットテストを複数回行うと、一般に漏れ率は徐々に大きくなる

チェックリスト結果

	チェックリスト
医師	11.7 ± 0.3
看護師	11.8 ± 0.3
全体	11.7 ± 0.3
（満点）	12

平均±標準偏差

チェックリストは前後の比較なし

＊　＊　＊

		できた	できない	配点	得点
1.　手指衛生					
1	手袋を外す際に汚染が起こり得ることを確認した（自分またはグループ内で）			1	
2	手洗い後に、汚染が残りやすい部位を確認した			1	
2.　吐物処理					
	チェックリストは記入不要	—	—	—	—
3.　PPE 装着					
3	ボディスーツ型ガウンを適切に装着した			1	
4	手袋を適切に装着した			1	
5	マスクを適切に装着した			1	
6	ゴーグルを適切に装着した			1	
7	靴カバーを適切に装着した			1	
4.　マネキン訓練					
8	ルート確保の際、針を安全に廃棄した（グループ評価）			0.5	
9	吐物処理を、汚染を封じ込めつつ遂行した（グループ評価）			0.5	
10	病衣交換を、患者に配慮しつつ実行した（グループ評価）			0.5	
11	心肺蘇生法を適切に実施した（グループ評価）			0.5	
12	一連の診療・ケア後の自分の汚染状況を確認した			1	
13	PPE を外して適切に廃棄した			1	
14	PPE を外した後、自分の体表に汚染を残さなかった			1	
総得点				12	

一類感染症ワークショップ　参加者アンケート結果

1. **あなたの職種は：**　□医師 10 名　□看護師 14 名

2. **講義：ウイルス性出血熱（加藤）**

	医師	看護師	全体
講師の説明は明確でしたか	2.70	2.79	2.75
内容は、あなたに関連したものでしたか	2.90	2.79	2.83
一類感染症に関する診療・看護に役立つと思いますか	2.90	2.86	2.88

※項目 2～10 は、「そう思う」3 点、「まあまあ」2 点、「そう思わない」1 点とした、3 点満点の平均値

3. **討論：臨床シナリオ検討（足立）**

	医師	看護師	全体
担当者の誘導は明確でしたか	2.70	2.79	2.75
内容は、あなたに関連したものでしたか	2.90	2.86	2.88
一類感染症に関する診療・看護に役立つと思いますか	2.80	2.79	2.79

4. **セミナー：新興ウイルス感染症の現況と我が国の臨床的対応を考える（西條）**

	医師	看護師	全体
講師の説明は明確でしたか	2.67	2.79	2.74
内容は、あなたに関連したものでしたか	3.00	2.50	2.70
一類感染症に関する診療・看護に役立つと思いますか	2.89	2.64	2.74

5. **セミナー：感染研におけるウイルス性出血熱患者発生に備えた検査診断対応（森川）**

	医師	看護師	全体
講師の説明は明確でしたか	2.44	2.57	2.52
内容は、あなたに関連したものでしたか	2.56	2.29	2.39
一類感染症に関する診療・看護に役立つと思いますか	2.56	2.43	2.48

6. **セミナー：Dangerous infections: risk management and clinical care（Bannister）**

	医師	看護師	全体
講師の説明は明確でしたか	2.89	2.71	2.78
内容は、あなたに関連したものでしたか	3.00	2.79	2.87
一類感染症に関する診療・看護に役立つと思いますか	3.00	2.86	2.91

7. **講義：院内における接触者調査（中島）**

	医師	看護師	全体
講師の説明は明確でしたか	2.90	2.79	2.83
内容は、あなたに関連したものでしたか	2.80	2.86	2.83
一類感染症に関する診療・看護に役立つと思いますか	2.90	2.79	2.83

8. **講義：職業安全保健（個人用防護具）（吉川）**

	医師	看護師	全体
講師の説明は明確でしたか	2.80	2.93	2.88
内容は、あなたに関連したものでしたか	2.90	2.93	2.92
一類感染症に関する診療・看護に役立つと思いますか	2.80	2.93	2.88

9. **実習：呼吸用保護具（吉川ほか）**

	医師	看護師	全体
担当者の指導は明確でしたか	2.80	3.00	2.92
内容は、あなたに関連したものでしたか	2.90	3.00	2.96
一類感染症に関する診療・看護に役立つと思いますか	2.80	3.00	2.92

10. **実習：手指衛生、吐物処理、個人用防護具装着、マネキン訓練（足立）**

	医師	看護師	全体
担当者の指導は明確でしたか	2.80	3.00	2.92
内容は、あなたに関連したものでしたか	2.90	2.93	2.92
一類感染症に関する診療・看護に役立つと思いますか	2.90	3.00	2.96

11. **このワークショップを 5 段階で評価すると：**

□非常によかった　□よかった　□まあまあ　□あまりよくなかった　□よくなかった

医師 4.50　看護師 4.50　全体 4.50

※「非常によかった」5 点、「よかった」4 点、「まあまあ」3 点、「あまりよくなかった」2 点、「よくなかった」1 点とした、5 点満点の平均値

12. **今回の内容以外にあった方がよい項目があれば、記載してください。**

（医師）

- 感染性廃棄物の処理。Ethics（患者の治療、生命維持について）
- スタッフのマネージメント（勤務交代、医師の勤務時間）。世界の例を挙げて
- 実習の DVD 学習など
- 一類感染症の支持療法中に生じた合併症に対し、どこまで対応すべきか。例えば手術など
- リネン、食器などの取り扱い。臨床例の紹介をもっと多く（写真など）
- 診療に用いた機器（例えば超音波、呼吸器等）の扱い。検体検査をどこで誰が行うか等、検査技師他にも情報提供できるように

（看護師）

- 看護ケアをより深めてシミュレーションしたい
- 新型インフルエンザ等、新興感染症対策。体制（職員、待遇、行政との関係）等
- 各施設のマニュアルやシミュレーションでの問題点を考え、教えあうような機会

13. **この企画についてのコメントを、自由に記載してください。**

（医師）

- こういう機会はとても良いと思う。今後も他病院との話し合いを具体的にできればと思った。ガイドライン

作成は助かる

- 接触予防に偏っていたかも
- ウイルス性出血熱についてのまとまった講義を聴くことができてよかった
- 数年に一度以上の頻度で、対象職種を拡大した形で開催されれば、実際のリスク縮小につながると思う
- 専門家の詳細な講義に加え、医師、看護師を交えた討議の機会があり、様々な意見を聞くことができ大変有意義であった。また実習では、普段のシミュレーションでは気づかなかった点について考える契機を与えられ、非常に役立ったと思う
- なかなかこういう風に勉強する機会がない中、バーバラ先生をはじめ一線のスペシャリストの講義が受けられ、大変ラッキーだった。西條先生のコメント通り、別の感染症にも応用が効くと思うので、今後に役立てたい
- 定期的に参加して、情報アップデートをしつつ、本当に当院第一例が来たときにあわてないように備えたい
- シナリオ検討について答えはないと思うが、グループにより考え方が異なっていたと思うので、その点についてつっこんで話し合う時間が欲しかった
- 討論、実習の時間を、もう少し取って欲しかった（実習後の討論時間を特に）。医療機関を監督している部門（都立なら東京都福祉保健局や保健所）の参加者を増やして、現場の声を知ってほしい。本気で実のある連携を考えて欲しい

（看護師）

- 一類感染病床を管理する師長としての意識が高まった。患者入室のシミュレーションは年１回実施しているが、内容を再考する必要があると感じた。訓練された医師、看護師、コメディカルが対応しなければならないことを痛感した。スペシャルチームが東北地方に何チームかあるとよい
- 他の一類を受け容れる施設と意見交換したり、専門家に実習をまじえた講義を受けるのは初めて。非常に有意義だった。引き続き参加したい。バーバラ先生の話している内容の説明をもう少し伝えていただきたかった
- 再度参加したい。日々の疑問が明らかになった。マニュアル改訂で入れるシミュレーションの内容がより具体的になった
- 医師、看護師がチームで参加したことはよかった。班分けも地域ごとにされていたが、地域内での連携や情報交換をすることも今後必要かと思う。個人的には一類や新興感染症は特別という意識はなく、通常から正しい感染対策・知識を持つことが重要と考えている
- 講義だけでなく実習があり、分かりやすく学ぶことができた。講師にとても丁寧に説明してもらい、有意義な研修だった
- 一類感染症受け容れ準備やマニュアル作成の際、情報が少なく非常に悩んでいた。実際に海外で起こった状況や、どのような人が曝露したか、またバーバラ先生の施設での対応方法を見たり聞いたりすることで想像できて、自分のアセスメントをする上での資料となった
- 一類指定病院の情報交換や、指示、相談のネットワークがあればよいと思う。事前に質疑内容を知らせていただければスムーズでは。マニュアルを相互にチェックできればよい
- 自施設での研修に応用できそうな実習内容でよかった。ただ、実際やってみると予想以上に難しく、訓練を受けたスペシャルチームを派遣して対応するほうが、院内アウトブレイク等を防げるのではないかと感じた
- バーバラ先生の講演はめったに聞くことができない機会でありがたかった。質問に対する訳をもっと分かりやすく言ってほしかった
- 定期的にこの企画をしていただきたい。もっと多くの医師、看護師に勉強してもらいたい。感染症は感染病棟スタッフだけでは対応しきれないので、もっと看護師の苦労も理解をしてもらえたらと思った

- 吐物処理訓練、定量フィットテストは、施設に帰って次年度実践しようと思う。グリッターバグの粉があるのを初めて知ったので、今後研修に取り入れたい。普段会えない同じ一類指定機関の方に会えて、とてもよい機会となった
- 予防衣の着脱方法など、他院でのマニュアル内容を知ることで、自施設の見直しが必要な点を洗い出すことができ、貴重な情報となった。実際にアウトブレイクが起こった（外国の）状況について、具体的に教えていただき、自施設でどこまで対応ができるのか、どこまでの対応が必要なのかなど、考える機会となった
- マネキン訓練、手指衛生など、施設に持ち帰り実践したい内容だった。PPE 選択や保管、メンテナンスについても考える良い機会となった

研究成果の刊行に関する一覧表

雑誌

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Reston Ebolavirus Antibodies in Bats, the Philippines

To the Editor: Filoviruses cause highly lethal hemorrhagic fever in humans and nonhuman primates, except for Reston Ebolavirus (REBOV), which causes severe hemorrhagic fever in macaques (*1*,*2*). REBOV epizootics among cynomolgus macaques occurred in 1989, 1990, 1992, and 1996 (*2*) and among swine in 2008 (*3*). African fruit bats have been suggested to be natural reservoirs for Zaire Ebolavirus and Marburg virus (*4*–*6*). However, the natural reservoir of REBOV in the Philippines is unknown. Thus, we determined the prevalence of REBOV antibody–positive bats in the Philippines.

Permission for this study was obtained from the Department of Environment and Natural Resources, the Philippines, before collecting bat specimens. Serum specimens from 141 wild-caught bats were collected at several locations during 2008–2009. The bat species tested are summarized in the Table. Captured bats were humanely killed and various tissues were obtained. Carcasses were then provided to the Department of Environment and Natural Resources for issuance of a transport permit.

We used immunoglobulin (Ig) G ELISAs with recombinant nucleoprotein (NP) and glycoprotein (GP) of REBOV (*7*) to determine REBOV antibody prevalence. REBOV NP and GP were expressed and purified from Tn5 cells infected with recombinant baculoviruses AcResNP and AcResGPDTM, which express NP and the ectodomain of GP with the histidine tag at its C-terminus. We also used histidine-tagged recombinant Crimean-Congo hemorrhagic fever virus NP as a negative control antigen in the IgG ELISA to confirm specificity of reactivity.

In IgG ELISAs for bat specimens, positive results were detected by using rabbit anti-bat IgG and horseradish peroxidase–conjugated anti-rabbit IgG. Anti-bat (*Rousettus aegyptiacus*) rabbit IgG strongly cross-reacts with IgGs of other bat species, including insectivorous bats (*8*). Bat serum samples were 4-fold serially diluted (1:100–1:6,400) and tested by using IgG ELISAs. Results of IgG ELISAs were the sum of optical densities at serum dilutions of 1:100, 1:400, 1:1,600, and 1:6,400. Cutoff values (0.82 for both IgG ELISAs) were determined by using serum specimens from REBOV antibody–negative bats.

Among 16 serum samples from *R. amplexicaudatus* bats, 5 (31%) captured at either the forest of Diliman (14°38′N, 121°2′E) or the forest of Quezon (14°10′N, 121°50′E) had positive results in the IgG ELISA for REBOV NP, and 5 (31%) captured at the forest of Quezon had positive results in the IgG ELISA for REBOV GP. The REBOV NP antibody–positive bats serum samples were confirmed to be NP antibody positive in the IgG ELISA by using glutathione-S-transferase–tagged partial REBOV NP antigen (*9*). Three samples had positive results in both IgG ELISAs (Table). Serum samples from other bat species had negative results in IgG ELISAs.

All bat serum samples were also tested by indirect immunofluorescence assays (IFAs) that used HeLa cells expressing NP and GP (*10*). In the IFAs, 2 samples from *R. amplexicaudatus* bats captured at the forest of Diliman and the forest of Quezon had high titers (1,280 and 640, respectively) of NP-specific antibodies, and 1 sample from an *R. amplexicaudatus* bat captured at the forest of Quezon had a positive result in the GP-specific IFA (titer 20). All IFA-positive samples were also positive in the IgG ELISA (Table).

The forest of Diliman is ≈30 km from the monkey facility and the Bulacan farm where REBOV infections in monkeys and swine, respectively, were detected. The forest of Quezon is ≈60 km from the monkey facility. Samples from other bat species had negative results in IFAs. We also performed heminested reverse transcription PCR specific for the REBOV NP gene with spleen specimens from all 16 *R. amplexicaudatus* bats but failed to detect any REBOV-specific amplicons.

REBOV-specific antibodies were detected only in *R. amplexicaudatus* bats, a common species of fruit bat, in the Philippines. In Africa, *R. aegyptiacus* bats, which are genetically similar to *R. amplexicaudatus* bats, have been

Table. REBOV-specific IgG in *Rousettus amplexicaudatus* bats and other bats, the Philippines*

Bat ID	Collection site	ELISA optical density		IFA titer	
		REBOV NP	REBOV GP	REBOV NP	REBOV GP
1539	FD	**2.13**	–0.21	**1,280**	<20
1632	FQ1	**0.88**	0.2	<20	<20
1642	FQ1	0.36	**5.22**	<20	**20**
1643	FQ1	**1.26**	**0.92**	<20	<20
1651	FQ1	**1.61**	**1.02**	<20	<20
1657	FQ1	–0.45	**1.69**	<20	<20
1660	FQ1	**3.8**	**2.51**	**640**	<20

*Cutoff optical density of ELISA was 0.82 (sum of optical densities at serum dilutions of 1:100, 1:400, 1:1,600, and 1:6,400). Values in **boldface** are positive results. REBOV, Reston Ebolavirus; Ig, immunoglobulin; IFA, indirect immunofluorescence assay; ID, identification; NP, nucleoprotein; GP, glycoprotein; FD, forest of Diliman at the University of the Philippines Diliman campus; FQ1, forest at the Agricultural College in Province of Quezon, the Philippines. The other 9 *R. amplexicaudatus* bats collected at FQ1 had negative results for all assays. The following bat species also had negative results: 5 *Eonycteris spelaea*, 35 *Cynopterus brachyotis*, 38 *Ptenochirus jagoli*, 6 *Haplonycteris fischeri*, 2 *Macroglossus minimus*, 2 *Rhinolophus rufus*, 1 *Rhinolophus arcuatus*, 9 *Emballonura alecto*, 2 *Pipistrellus javanicus*, 5 *Scotophilus kuhlii*, 8 *Miniopterus australis*, 8 *M. schreibersi*, 1 *M. tristis tritis*, 1 *Hipposideros diadema*, 1 *Myotis macrotarsus*, and 1 bat of unknown species.

shown to be naturally infected with Zaire Ebolavirus and Marburg virus. Thus, *R. amplexicaudatus* bats are a possible natural reservoir of REBOV. However, only 16 specimens of *R. amplexicaudatus* bats were available in this study, and it will be necessary to investigate more specimens of this species to detect the REBOV genome or antigens to conclude the bat is a natural reservoir for REBOV.

We have shown that *R. amplexicaudatus* bats are putatively infected with REBOV or closely related viruses in the Philippines. Antibody-positive bats were captured at the sites near the study areas, where REBOV infections in cynomolgus monkeys and swine have been identified. Thus, bats are a possible natural reservoir of REBOV. Further analysis to demonstrate the REBOV genome in bats is necessary to conclude that the bat is a reservoir of REBOV.

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Acute Hepatitis C Outbreak among HIV-infected Men, Madrid, Spain

To the Editor: In the past decade, hepatitis C virus (HCV) has emerged as a sexually transmitted infection (STI) among HIV-infected men who have sex with men (MSM). The epidemic was originally reported in several northern European countries (England, France, Germany, and the Netherlands) (*1*) and soon after in Australia (*2*) and the United States (*3*). Acute HCV acquisition was associated with group sex, unprotected receptive anal intercourse, and according to some studies, concomitant STI (*4*). Molecular phylogenetic studies suggested evidence of an international transmission network of MSM within northern Europe (*1*). However, expansion of the HCV epidemic among MSM to Spain (*5*) or to other

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Antigen-capture ELISA for the detection of Rift Valley fever virus nucleoprotein using new monoclonal antibodies

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Monoclonal antibodies (MAbs) raised against the nucleoprotein (NP) of Rift Valley fever virus (RVFV) were developed, and an antigen-capture enzyme-linked immunosorbent assay (Ag-capture ELISA) system was developed for the detection of RVFV NP. The assay detected RVFV antigen from culture supernatants containing as little as 7.8–31.3 pfu per 100 μl. Reactivity with various truncated NPs indicated that MAb C10-54 bound only to the full-length NP, probably due to recognition of a conformational epitope, whereas MAbs G2-36 and D5-59 bound to a linear epitope ranging from amino acid residues 195–201 in the C-terminal region. Based on the alignments of the amino acid sequence of RVFV NP, the epitope regions of MAbs G2-36 and D5-59 were completely conserved among all RVFV strains. These results suggest that the MAbs are applicable to the Ag-capture ELISA for the diagnosis of RVFV infections.

1. Introduction

Rift Valley fever (RVF), a mosquito-borne zoonotic disease that affects domestic animals and humans, is caused by infection by the RVF virus (RVFV). The disease is found in sub-Saharan areas in Africa, as well as in Egypt, the Comoros Islands, Madagascar, and the Arabian Peninsula (Shimshony and Barzilai, 1983; Shoemaker et al., 2002; Sissoko et al., 2009). Infection of RVFV causes abortions or resorption of the fetus in pregnant domestic ruminants, with newborn mortality approaching 100%, and thus can cause catastrophic economic losses. Transmission of RVFV to humans, either through contact with bodily fluids of infected animals or mosquito bites, may result in mild to moderate influenza-like symptoms and severe retinitis, encephalitis and hemorrhagic fever (Alrajhi et al., 2004; Gerdes, 2004; Shimshony, 1999). During an RVF outbreak, confirmed cases are defined as suspected or probable cases by laboratory confirmation of the presence of anti-RVFV IgM by enzyme-linked immunosorbent assay (ELISA), RVFV antigen by antigen-captured (Ag-captured) ELISA, or viral RNA by RT-PCR in serum or blood samples (Al-Hazmi et al., 2003; Bird et al., 2008; Madani et al., 2003; MMWR, 2007).

RVFV belongs to the *Phlebovirus* genus of the *Buniyaviridae* family. Like other members of the family, RVFV possesses a single-stranded tripartite RNA genome composed of three segments, namely, S, M, and L. The S segment codes for nucleocapsid protein (NP) in negative sense, and non-structural NSs protein in positive sense, using an ambisense strategy. The M segment codes for a precursor of glycoproteins Gn and Gc and two non-structural proteins of 78 kDa and 14 kDa. The L segment codes for an L protein. The nucleotide sequence of the NP gene is highly conserved among various RVFV isolates (Bird et al., 2007b). Serum antibodies against NP are detected readily early after infection and in the convalescent phase, providing a basis for the diagnosis of RVF (Fafetine et al., 2007; Jansen van Vuren et al., 2007). An Ag-capture ELISA for detecting viral NP has been applied commonly to detect RVFV, as well as various viruses, since it is the most abundant viral antigen (Al-Hazmi et al., 2003; Bird et al., 2008; Jansen van Vuren and Paweska, 2009; Ji et al., 2011; Saijo et al., 2005, 2006, 2007; Madani et al., 2003; Nakauchi et al., 2009; Velumani et al., 2008). Monoclonal antibodies (MAbs) are used often as a capture antibody for Ag-capture ELISA since they have a high specificity for antigens, and identification of the epitopes of MAbs is of crucial importance for the assessment of cross-reactivity of the assay (Saijo et al., 2005, 2006, 2007; Nakauchi et al., 2009). In this study, MAbs were raised against recombinant RVFV NP (RVFV rNP). Epitope mapping showed that these MAbs recognized highly conserved epitopes on RVFV NP, suggesting their potential application for the detection of all RVFV isolates. By using these MAbs as capture antibodies, an Ag-capture ELISA for detecting an RVFV antigen was developed.

2. Materials and methods

2.1. Recombinant baculoviruses

Ac-His-RVFV-NP baculoviruses, expressing RVFV rNP, were generated using the same strategy as previously described (Saijo et al.,

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2002). Briefly, an entire cDNA clone of NP from RVFV-MP12 was used to construct a transfer vector. RVFV NP cDNA was amplified by PCR. The amplified DNA was digested with BamHI and subcloned into the BamHI site of pQE30 vector DNA (QIAGEN GmbH, Hilden, Germany) to construct pQE30-RVFV NP. An RVFV NP DNA fragment with a six-histidine (His) tag was isolated from the pQE30-RVFV NP plasmid by digestion with EcoRI and SalI. Each extremity was then blunted with T4 DNA polymerase and subcloned into the blunt-ended BamHI site of pAcYM1 (Saijo et al., 2002) to generate pAcYM1-His RVFV rNP.

Tn5 insect cells were transfected with mixtures of linearized BacPAK6 DNA (Clontech, Mountain View, CA) and the recombinant transfer vector pAcYM1-His RVFV rNP according to the manufacturer's instructions and the procedures described by Kitts and Possee (1993). The resulting recombinant baculovirus, which expresses a His-tagged recombinant NP of RVFV (His-RVFV rNP), was designated as Ac-His-RVFV NP.

2.2. Expression and purification of rNPs

Ac-His-RVFV NP-infected Tn5 cells were incubated at 26 °C for 72 h. Then, the cells were washed twice with cold phosphate-buffered saline (PBS) solution and lysed in cold PBS solution containing 1% Nonidet P-40 (NP-40). The cell lysate was centrifuged at 13,000 × *g* at 4 °C for 10 min. The supernatant fraction was collected as a source of His-RVFV rNP for purification. The His-RVFV rNP was purified by Ni^{2+} column chromatography (QIAGEN GmbH), as previously described (Saijo et al., 2002). Sabia virus (SABV) rNP as a control was expressed and purified, as described previously (Nakauchi et al., 2009).

2.3. Establishment of MAbs

BALB/c mice were immunized subcutaneously three times with the purified His-RVFV rNP. Spleen cells were obtained 3 days after the last immunization and fused with P3/Ag568 cells using polyethylene glycol (Invitrogen). The culture supernatants of the hybridoma cells were screened by ELISA with purified His-RVFV rNP as an Ag in the presence of 2 M urea in order to exclude MAbs with a low-avidity. MAbs, designated as D5-59, C10-54, and G2-36, were purified from the culture supernatants of the respective hybridoma clones using protein-G column chromatography, as described previously (Nakauchi et al., 2009).

2.4. Polyclonal antibodies

Polyclonal antibodies were raised in rabbits by immunization with the purified His-RVFV rNP expressed in the baculovirus system, as described previously (Saijo et al., 2002). Protocols for animal experiments were approved by the Animal Care and Use Committee of the National Institute of Infectious Diseases, Tokyo, Japan.

2.5. Indirect immunofluorescence assay

Vero E6 cells were infected with RVFV-MP12 (MOI = 0.1). After 18 h, the cells were fixed with a mixture of methanol and acetone [1:1 (v/v)]. Binding of the RVFV infected cells was performed by immunofluorescence assay (IFA), as described previously (Saijo et al., 2005).

2.6. Ag-capture ELISA

The Ag-capture ELISA was performed essentially as described elsewhere (Saijo et al., 2005, 2006, 2007; Nakauchi et al., 2009). Purified MAb D5-59, C10-54, or G2-36 was coated on 96-microwell immunoplates (Falcon; Becton Dickinson Labware) at 100 ng/well in 100 μl of PBS at 4 °C overnight, followed by blocking with PBS containing 0.05% Tween-20 and 5% skim milk (PBST-M) for 1 h at room temperature. After the plates were washed three times with PBS containing 0.05% Tween-20 (PBST), 100 μl samples containing serially diluted His-RVFV rNP or culture supernatants of Vero cells, either infected with RVFV MP12 or uninfected, were added, and the plates were incubated for 1 h at 37 °C (viruses were inactivated by treatment with 1% NP40 followed by UV irradiation for 15 min). The plates were then washed with PBST, and 100 μl of rabbit anti-serum raised against His-RVFV rNP diluted 1:500 with PBST-M was added to each well. After 1 h of incubation at 37 °C, the plates were washed with PBST, and horseradish peroxidase-conjugated goat anti-rabbit IgG (Zymed, San Francisco, CA) was added. The plates were further incubated for 1 h at room temperature. After another extensive washing with PBST, 100 μl of ABTS substrate solution (Roche Diagnostics) was added and the OD405 was measured with a reference wavelength of 490 nm after 30 min of incubation at room temperature. As a negative control, the ODs of wells inoculated with control Ag (SABV rNP or culture supernatants of mock-infected Vero cells) were measured. Means and standard deviations were calculated from the OD405 values of 12 negative control wells, and the cutoff value for the assay was defined as the mean plus three standard deviations.

2.7. Expression of truncated rNPs of RVFV

In order to determine the epitope region reacted with the MAbs, a series of truncated RVFV rNPs were expressed as fusion proteins with glutathione S-transferase (GST), as previously described (Nakauchi et al., 2009). Briefly, the cDNA corresponding to each of the truncated NP fragments was amplified by PCR with specifically designed primer sets. The amplified DNA was subcloned into the BamHI and EcoRI cloning sites of plasmid pGEX-2T (Amersham Pharmacia Biotech, Buckinghamshire, England). The GST-tagged full-length RVFV NP or truncated forms of RVFV NP were expressed in *Escherichia coli* (*E. coli*) BL21.

2.8. Western blotting

The MAbs were tested for reactivity to the GST-tagged RVFV NP fragments expressed in *E. coli* by Western blotting, as described previously (Saijo et al., 2005). Goat anti-GST antibody (GE Healthcare, Piscataway, NJ) was used for detection of GST-tagged proteins in the assay.

2.9. Plaque assay

VeroE6 cells prepared in 12-well plates were inoculated with 50 μl of 10-fold serially diluted virus samples and incubated at 37 °C for 1 h. Then the cells were cultured with 1.0 ml per well of DMEM containing 1% FCS and 1% methyl cellulose (Sigma) for 5 days. Cells were fixed with 10% formalin, irradiated under a UV lamp, and stained with crystal violet. Plaques produced by RVFV were counted under light microscopy. Titration was done in duplicate and infectivity was displayed by plaque-forming units (pfu).

3. Results

3.1. Generation of MAbs

In order to obtain MAbs against RVFV NP, BALB/c mice were immunized with purified RVFV rNP. The hybridomas were cloned and their culture supernatants were tested for reactivity to RVFV rNP by IgG ELISA. Three MAbs, designated D5-59, C10-54, and G2-36, reacted with the His-RVFV rNP by IgG ELISA, even in the presence of 2.0 M urea (data not shown). The reactivity of MAbs

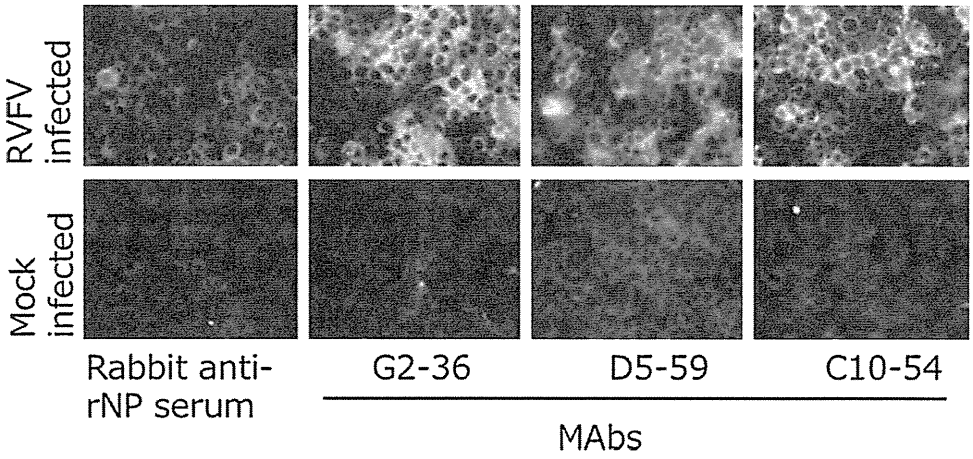

Fig. 1. Reactivity of MAbs in IFA. Vero E6 cells infected with RVFV MP12 were stained by indirect immunofluorescence with MAbs D5-59, C10-54, and G2-36. Rabbit anti-rNP serum was used as a positive control.

was examined by an indirect immunofluorescence method (Fig. 1). The RVFV-infected (MOI = 0.1), but not mock-infected, cells were stained with each of these MAbs (Fig. 1). The staining pattern was consistently similar in all MAbs tested and was characterized by a diffuse granular cytoplasmic staining, similar to that observed previously (Billecocq et al., 1996), indicating specific recognition of MAbs against RVFV NP.

3.2. Development of Ag-capture ELISA

By using these MAbs and rabbit anti-rNP serum as capture and detection antibodies, respectively, an Ag-capture ELISA for detecting RVFV NP was developed. When the His-RVFV rNP antigen was used in Ag-capture ELISA, MAbs D5-59 and G2-36 detected as little as 0.16 ng per 100 μl of rNP, whereas MAb C10-54 was more sensitive in detecting the rNP, with a detection limit of 0.08 ng per 100 μl (Fig. 2). None of these MAbs reacted with rNP of the control virus, SABV, prepared from insect cells, even at high antigen concentration (20 ng per 100 μl). RVFV antigen in the culture supernatants from Vero E6 cells infected with RVFV-MP12 was also detected in the developed Ag-capture ELISA, whereas mock-infected cells showed a negative reaction, indicating that MAbs reacted not only with recombinant NP, but also with an authentic viral NP (Fig. 3). MAbs D5-59, C10-54, and G2-36 were able to detect as little as 15.6, 7.8, and 31.3 pfu per 100 μl of RVFV, respectively.

3.3. Epitope mapping of MAbs

To determine the binding regions including epitopes of MAbs, truncated NPs were expressed in *E. coli* and analyzed for MAbs reactivity by Western blot analysis. At first, five forms of truncated rNP, as well as full-length rNP, were examined for MAbs reactivity (Fig. 4A). MAbs G2-26 and D5-59 reacted with the full-length (NP1-245) and C-terminus region (NP177-245 and NP76-245) of rNP, whereas MAb C10-54 reacted only with the full-length rNP. These results indicated that the binding region for MAbs G2-36 and D5-59 mapped within the C-terminus one-third, corresponding to amino acid residues 177–245, and that full-length RVFV NP was required for MAb C10-54 to react. MAb C10-54 could recognize a conformational epitope since it reacts weakly by Western blot against full-length rNP, probably due to epitope renaturation during or after the transfer of the protein to a membrane as reported by Zhou et al. (2007).

To narrow the region recognized by MAbs G2-36 and D5-59, additional truncated rNPs from amino acid residues 177–245 were generated and tested for reactivity by Western blot analysis. Both MAbs reacted with NP177-201 and NP195-235, but not with NP177-200 and NP196-235, indicating that minimum region for these MAbs ranged from amino acid residues 195–201 (Fig. 3B). The results also suggested essential amino acid residues 195 and 201 for MAb binding. Although one truncated NP (NP177-200) was reacted with MAbs G2-36 and D5-59, the intensities of this protein bands were significantly lower than those of the NPs containing amino acid residues 195–201. The result might be attributed to the lack of critical amino acid residue 201 on NP177-200.

To ascertain whether MAbs G2-36 and D5-59 react broadly with various RVFV isolates, the amino acid sequence of the minimum epitope region ($_{195}$TFTQPMN$_{201}$) was aligned with corresponding amino acid sequences of all known RVFV isolates, as well as those of other Phleboviruses, deposited in the GenBank database (Fig. 4). The amino acid sequence ($_{195}$TFTQPMN$_{201}$) of the epitope was conserved completely among all RVFV isolates, but was not identical to those of other Phleboviruses. These results demonstrated that MAbs G2-36 and D5-59 recognized a highly conserved linear epitope in the RVFV NP.

4. Discussion

In diagnosing many virus infections, PCR assays have excellent analytical sensitivity, but the established techniques are limited by the need for expensive equipment and technical expertise. Since the sensitivity of Ag-capture ELISA is potentially comparable to that of RT-PCR (Ji et al., 2011; Saijo et al., 2006, 2007; Velumani et al., 2008), Ag-capture ELISA represents a sophisticated approach for the diagnosis of virus infections. As a prelude to the development of such a sensitive diagnostic test for RVFV infection, a recombinant RVFV NP from insect cells infected with recombinant baculovirus was purified and novel MAbs against them were developed. MAbs D5-59, G2-36, and C10-54 reacted with His-RVFV rNP and authentic viral antigen (NP) in RVFV MP12-infected cells. Furthermore, control assays with unrelated virus (SABV) rNP and mock-infected cells revealed that MAbs were specific to RVFV NP.

Although it is difficult to compare simply the detection limits among the various assay procedures, the Ag-capture ELISA developed in this study seems to have excellent sensitivity. The detection limit of the newly developed Ag-capture ELISA (0.08–0.16 ng of rNP per 100 μl, Fig. 2) was very similar to that of a previous report, in which an *E. coli* system for the expression of RVFV rNP was used, hyperimmune sheep anti-RVFV rNP serum was used as the capture antibody, and the detection limit of the assay was 0.11 ng per 100 μl of rNP (Jansen van Vuren and Paweska, 2009).

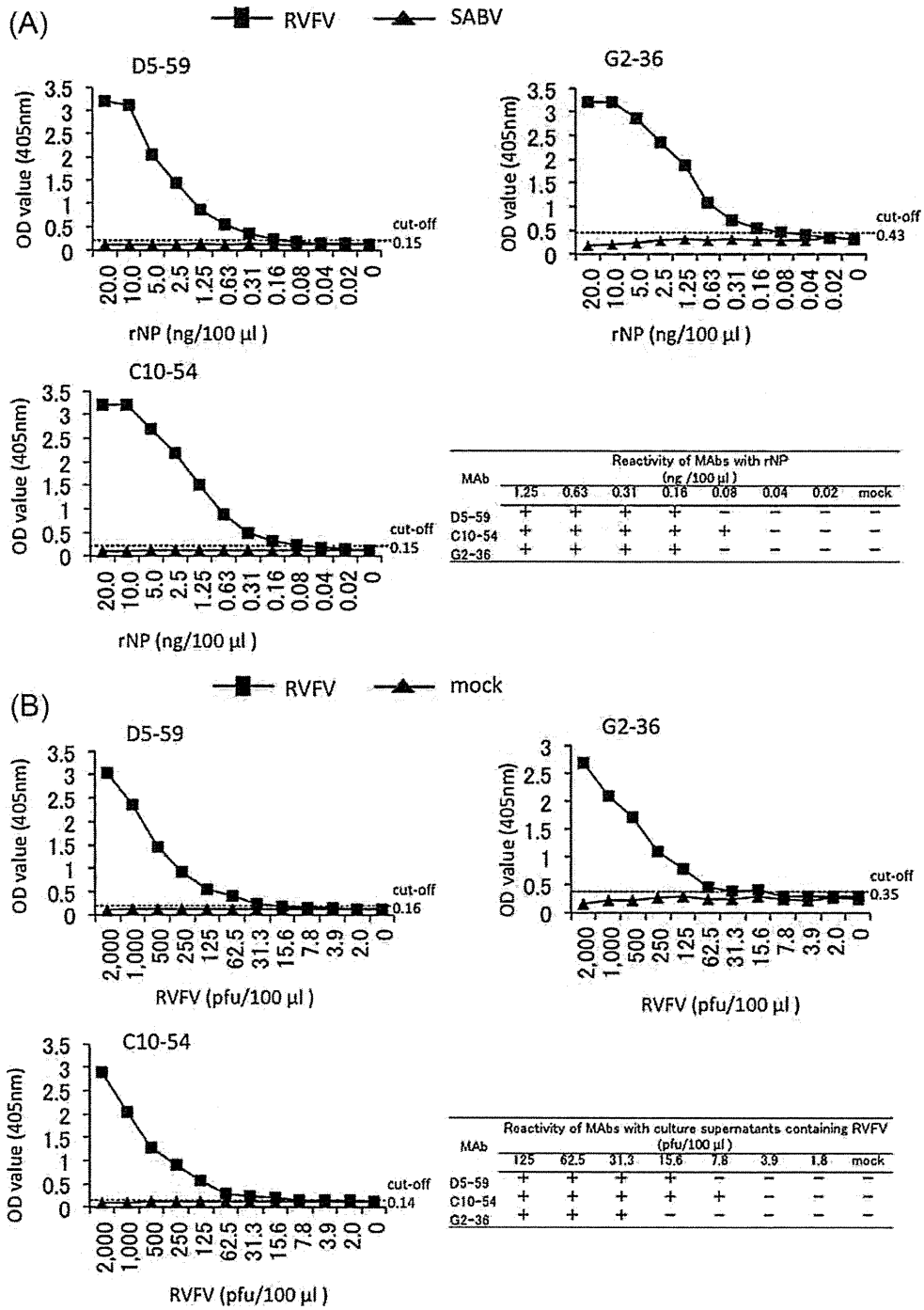

Fig. 2. Ag-capture ELISA for the detection of His-RVFV rNP (A) and authentic RVFV NP (B). MAbs D5-59, C10-54, and G2-36 were used as capture antibodies. Rabbit anti-rNP serum was used as the detecting antibody. Dashed lines indicate the cut-off for each antibody. Detection limits for each MAb are summarized in the table.

Using different amounts of authentic RVFV antigen obtained from the culture supernatants of cells infected with RVFV, as little as 7.8–31.3 pfu per 100 µl of RVFV was detected by ELISA. A real-time RT-PCR assay (Bird et al., 2007a) for detection of the RVFV genome from the same culture supernatant samples detected as little as 3.0 pfu per 100 µl (data not shown), indicating that the detection limit of the Ag-capture ELISA was slightly less than that of the real-time RT-PCR assay. However, the detection limit of this Ag-capture ELISA was approximately 10 times higher than that reported for detecting authentic RVFV antigen (Jansen van Vuren and Paweska, 2009). Thus, the newly developed Ag-capture ELISA might be useful in the diagnosis of RVFV infection.

MAbs directed against RVFV NP and their application for detecting RVFV have been reported, showing broad reactivity to RVFV strains (Martin-Folgar et al., 2010; Saluzzo et al., 1989). Since MAbs directed against RVFV NP might allow for the detecting RVFV antigen in the serological diagnosis, identification of the epitopes of MAbs is of crucial importance for the assessment of specificity of the assay system.

The Ag-capture ELISA using MAb C10-54 recognizing a conformational epitope on RVFV NP proved more sensitive than assays using MAbs D5-59 and G2-36 recognizing the linear epitope, indicating that, similar to the results shown by another diagnostic study (Velumani et al., 2008), conformation-specific MAb might