

Dilution of BSE into scrapie brain homogenate							
BSE isolate	Scrapie isolate	1 in 50	1 in 250	1 in 1600	1 in 6000	1 in 31000	1 in 166000
Caprine BSE							
ARQ/AHQ ^a		2/2	2/2	2/2	1/2	0/2	0/2
ARQ/AHQ ^a	ARQ/ARQ ¹	1/2	1/2	1/2	0/2	0/2	0/2
ARQ/AHQ ^a	ARQ/ARQ ²	2/2	1/2	1/2	1/2	0/2	0/2
ARQ/AHQ ^a	VRQ/VRQ ³	2/2	2/2	2/2	1/2	0/2	0/2
ARQ/AHQ ^a	ARQ/AHQ ^{CH}	2/2	2/2	1/2	2/2	0/2	0/2
ARQ/AHQ ^a	ARQ/AHQ ^{At}	2/2	1/2	0/2	0/2	0/2	0/2
ARQ/AHQ ^b		2/2	2/2	2/2	1/2	0/2	0/2
ARQ/AHQ ^b	ARQ/ARQ ¹	2/2	2/2	1/2	2/2	0/2	0/2
ARQ/AHQ ^b	ARQ/ARQ ²	2/2	0/2	1/2	0/2	0/2	0/2
ARQ/AHQ ^b	VRQ/VRQ ³	2/2	2/2	2/2	2/2	0/2	0/2
ARQ/AHQ ^b	ARQ/AHQ ^{CH}	2/2	2/2	2/2	2/2	0/2	0/2
ARQ/AHQ ^b	ARQ/AHQ ^{At}	2/2	2/2	2/2	0/2	0/2	0/2
Ovine BSE							
ARQ/ARQ ^c		2/2	2/2	2/2	2/2	0/2	0/2
ARQ/ARQ ^c	ARQ/ARQ ³	2/2	2/2	1/2	0/2	0/2	0/2
ARQ/ARQ ^c	ARQ/ARQ ⁴	2/2	2/2	1/2	0/2	0/2	0/2
ARQ/ARQ ^c	VRQ/VRQ ⁵	2/2	2/2	1/2	0/2	0/2	0/2
ARQ/ARQ ^c	ARQ/AHQ ^{CH}	2/2	2/2	2/2	0/2	0/2	0/2
ARQ/ARQ ^c	ARQ/AHQ ^{At}	2/2	0/2	0/2	0/2	0/2	0/2
AHQ/AHQ ^d		2/2	1/2	2/2	1/2	0/2	0/2
AHQ/AHQ ^d	ARQ/ARQ ³	2/2	1/2	0/2	0/2	0/2	0/2
AHQ/AHQ ^d	ARQ/ARQ ⁴	2/2	1/2	0/2	0/2	0/2	0/2
AHQ/AHQ ^d	VRQ/VRQ ⁵	2/2	1/2	1/2	1/2	0/2	0/2
AHQ/AHQ ^d	ARQ/AHQ ^{CH}	2/2	1/2	2/2	2/2	0/2	0/2
AHQ/AHQ ^d	ARQ/AHQ ^{At}	2/2	2/2	2/2	0/2	0/2	0/2

Table 1S: Two different isolates of both caprine (samples a and b) and ovine (samples c and d) BSE (10% w/v brain homogenates) were either amplified either directly or diluted into either caprine or ovine scrapie brain homogenate as indicated, and then subjected to 5 days of sPMCA. Products from these reactions were detected by SHa31 western blot. Recorded are the numbers of positive reactions from a duplicate sPMCA test at each BSE dilution. Scrapie samples 1 and 2 were from caprine infected brain, whilst 3, 4 and 5 were of ovine origin. Scrapie brain denoted by CH was an ovine CH1641 isolate and that denoted At was an atypical ovine isolate.

BSE sample	Dilution of 10% w/v BSE in background of scrapie brain homogenates					
	1 in 50	1 in 250	1 in 1600	1 in 6000	1 in 31000	1 in 166000
a (caprine)	5/5	5/5	4/5	3/5	0/5	0/5
b (caprine)	5/5	4/5	5/5	3/5	0/5	0/5
c (ovine)	5/5	4/5	4/5	0/5	0/5	0/5
d (ovine)	5/5	5/5	3/5	2/5	0/5	0/5
% positive analyses	100%	90%	80%	40%	0%	0%

Table 2S: summary of sPMCA analyses of caprine and ovine BSE samples diluted into scrapie brain homogenates. Using the scoring system where a positive amplification is recorded if either 1 or 2 reactions in a duplicate analysis are positive in western blot, the assay demonstrates 100, 90, 80 and 40% sensitivity when BSE brain is diluted at 1/50, 1/250, 1600 and 1/6000 respectively in scrapie positive brain.