

Development of an enzyme-linked-immunosorbent-assay technique for accurate identification of poorly preserved silks unearthed in ancient tombs

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Abstract We developed a sensitive and specific enzyme-linked immunosorbent assay (ELISA) for the identification of poorly preserved silks. The method was based on the use of a monoclonal antibody (mAb) against the amino acid sequence "GAGAGSGAGAGS" of Bombyx mori silk fibroin. The mAb was prepared by immunizing mice with a recombinant protein (rBmSF) containing the target sequence. The specificity and sensitivity of the mAb were evaluated by Western blotting and ELISA. The results showed that the mAb could specifically recognize the target sequence in the silk fibroin samples. The detection limit of the mAb was 10 ng/ml. The method was applied to the identification of poorly preserved silks unearthed in ancient tombs. The results showed that the mAb could accurately identify the silks. The method is simple, sensitive, and specific, and can be used for the identification of poorly preserved silks.

a a . . . ca a . . . ac e^w, e e . . . a ed . . . e . . . ab . . .
 e . . . e- ed . . . be . . . a a (ELISA) . . . ca ace-
 . . . ace Te dea . . . e d c e a-
 . . . ce ce . . . e^w. S ce a . . . ae . 1990, e e a
 . . . de a e . . . ed . . . ec . . . de . . . e e ce
 . . . ac ae . . . ca . . . ec e ed b e, a d . . . ace b d
 e . . . e . . . d . . . a . . . ac . . . e e a a b de . . . a . [8, 9].
 I . . . ce . . . ea . . . ec . . . e a bee . . . a . . . ed . . . e
 . . . d . . . e . . . ace . . . b de . . . (c . . . d . . . c . . . ce -e . . . ,
 a b . . . e . . . , a d a . . . a . . . e) . . . ace . . . a . . . [10, 11].
 S . . . e e e a ce . . . a e e ce ed . . . e ELISA . . . e d a a
 . . . e . . . e a . . . ec . . . e . . . e . . . ae . . . ea . . . ca . . .
 . . . a d ce . . . c a . . . e . . . b d- ba ed . . . ae a . . . ad . . . a
 C . . . ee . . . a [12]. Te b d . . . ae a . . . ace . . . C . . . ee
 . . . e . . . e a ea bee . . . e . . . a ed b e ELISA . . . e -
 d, a d . . . e . . . e . . . e . . . a . . . e . . . e . . . a b . . . a d
 c . . . a e I . . . e . . . e ca e a ce b d . . . e . . . a d c e
 be ee be [13]. U . . . e . . . c . . . c . . . e^w, c ca . . . be
 . . . ed . . . a e . . . ec . . . a . . . e . . . be . . . a . . . e . . . e . . . e, ELISA
 . . . de . . . ec c de . . . ca . . . de a . . . ed . . . a . . . e . . . Ad-
 a ce . . . e a . . . ed . . . e de e . . . e . . . e . . . e -
 d . . . d . . . e . . . c . . .
 S . . . be . . . a ec . . . ed . . . a b . . . e . . . (b . . .) c e
 a d a . . . d . . . d . . . e . . . e . . . (e c) [14, 15]. Se c
 e . . . ed . . . e . . . ea . . . ce . . . , ea . . . b . . . a . . . e a
 c . . . e . . . [16]. S . . . b a e . . . e . . . e, b . . . a
 . . . c . . . a . . . a e a . . . e . . . e a . . . a . . . a . . . A a a e
 . . . e . . . , b . . . c a ea . . . ca . . . , . . . ca . . . , a d P25
 . . . e [17]. Te ea . . . ca . . . ade . . . a . . . e^w-c . . . e . . .
 e . . . b de ed b N a d C- e . . . a e . . . e . . . Te
 b . . . e . . . e^w-c . . . e . . . e . . . (4754 e d e) . . . ade
 e . . . ea . . . a GX d e . . . de . . . e^w, e e X A a . . . 65 %, . . .
 Se 23 %, a d T 10 % . . . e e e a c . . . a ed . . . a
 e d e . . . b . . . Te GX a e d . . . b . . . ed 12 d a . . . ,
 de . . . a ed GX d a . . . , 39 . . . 612 a . . . ac d e a a ed
 b 11 ea . . . de . . . ca c e . . . ab . . . d a . . . e . . . e ce . . . M . . .
 . . . e GX d e . . . de . . . a e . . . e e . . . a . . . a . . . e^w
 e a e . . . de GAGAGS (432 c e) a d GAGAGY (120
 c e) . . . c . . . e . . . e acc 72 % . . . e^w -
 c . . . e . . . e . . . X- a d ac . . . a e . . . be
 d ca e . . . a . . . e b e . . . e . . . e . . . a β- ee
 a e . . . (A a- G . . .). Eac GX d a c . . . d
 c . . . e a . . . e β- ee, a d . . . e b . . . d a . . . e . . . e ce b ea
 . . . e β- a d, e ab . . . e . . . e . . . e de ca . . . c a ed ec-
 [3, 18]. Te e e e . . . e β- ee c . . . a . . . e e . . . GX
 d a . . . a . . . e ca ace . . . c e . . . e . . . e be a d a
 d ce ca e . . . [5, 19].
 B e . . . e^w . . . ced . . . e . . . c . . . d e d . . . a . . . e . . . Te . . .
 a de c be . . . e . . . e d a d . . . de e . . . e . . . ,
 c . . . d . . . e . . . e a a e b . . . - e a b d a d
 de e . . . a e a d e ec . . . , a d . . . e . . . e -
 ca e . . . ab a a . . . ca e . . . d . . . e de -
 . . . ca a . . . a . . . e . . . I . . . e ec d a . . . , e . . . ed . . . e

a . . . b d . . . de a . . . e . . . e . . . ea . . . ed . . . a ce b
 d e e . . . e . . . e d . . .

Materials and methods

Choice and preparation of assay

Te a . . . -ac d e . . . e ce . . . e . . . b . . . ea . . . c a
Bombyx mori (E ec . . . c S . . . e e a . . . Ma e a (ESM)
 F . . . S1) . . . a . . . e e ed . . . e . . . NCBI . . . b c da aba e
 (<http://www.ncbi.nlm.nih.gov/pubmed/>). S . . . e e e . . . e a d
 c . . . e ed a . . . -ac d e . . . e ce (Tab e 1) . . . e e e e a ed
 . . . e . . . e^w . . . a e DNA a (L . . . B . . . , Sa Ra . . . ,
 USA). Te e . . . e ce "GAGAGSGAGAGS" . . . a . . . e ec ed a
 . . . ea . . . e . . . -a . . . b d . . . e a a . . . beca . . . e . . . ad . . . e
 . . . e . . . e . . . e ce . . . cc . . . e ce . . . b . . . Te . . . e
 . . . e . . . de "CGAGAGSGAGAGS"

50 L⁻¹ PBS (H 7.4) a a^w 60 L⁻¹. A. e⁴
 (10 L) a d⁴ ed^w 10 L 50 L⁻¹ PBS (H 7.4)
 a d^w a a ed a a^w 60 L⁻¹. T e a b d
 a e⁴ ed^w 0.1 L⁻¹ c e-HCL (H 3.0).
 D ec ELISA^w a e ed de e e e⁴
 d⁴ e ad e da e (HRP)-c⁴ a ed a
 a - abb. I G (ec da a b d, Abca, Ca b d e,
 UK) ⁴. T e a b d (a a b d) c a
^w a d⁴ ed c a b⁴ e (0.015 L⁻¹ Na₂CO₃,
 0.035 L⁻¹ NaHCO₃). a a c ce a⁸,
 4, 2, 1, a d 0.1 μ L⁻¹. T e ec da a b d c⁴ba-
^w a d⁴ ed b c⁴ (PBS, 1% BSA (S a,
 Ca a, USA)). 1:800, 1:1600, 1:3200, 1:5000, a d 1:10,
 000. I d ec ELISA^w a e ed de e e e⁴
 d⁴ e a -a b d⁴. P⁴ e b
 c a^w a d⁴ ed c a b⁴ e a a c ce a-
 8, 4, 2, 1, 0.5, 0.25, a d 0.125 μ L⁻¹. T e a
 a b d c⁴ba^w a d⁴ ed b c⁴
 1:500, 1:1000, 1:2000, 1:5000, a d 1:10,000. T e ec da
 a b d c⁴ba^w a d⁴ ed b c⁴
 1:5000 (ee "T e de a da c a a ed a e"
 ec ab⁴ ELISA ced⁴ e).

ed b ce⁴ a 5 a 12,000g. b a
 e⁴ e a a c a .
Testing of model and artificially aged samples
 T e a c a a ed a e^w e e e a a e e ac ed.
 A c a -a ed- a e e ac (100 μL^w) e e added
 c a ed e (G e e B -O e G bH, F c e a⁴ e, Ge-
 a) e a a e a d c⁴ba ed e a 4 C. A^w e
 e e a ed, a d⁴ e e e e ed a^w a
 4 4 e ced⁴ e e e e ed ee e (2
 e000098(516)]TJ0-1.2518999516Tc[(e000098())99878(G-1.2518
 23()0()13.1999(e)0()19.2000000318.899()-186.3999939

Samples and optimization of extraction

A. ca-a be a e c⁴ded (a a e
 2), e (a a e 3), c... (a a e
 3), a d^w (a a e 4), a d^w e e ded
 b e Mae a a d T e e I e Z e a Sc-Tec
 U e (Ha⁴, C a). T e e e e a a e⁴ b ec-
 ed d e a a e (125 C) 20 da ,
 e e a⁴ e a d⁴ d a e a c ed c a be
 (100 C, 100 % RH) 20 da , a d⁴ -e⁴ a e
 a e 20 da (e de a ab⁴ a c d ,
 ee Re .[3]).

F⁴ d e e a c a e ca a e (a a e 2
 eac^w) e e b a ed e C e e T e e I de ca
 a d C e a C e e C a S M⁴ e⁴. T e da ed
 bac 400 BCE, 0 CE, 400 CE, a d 1000 CE,
 e a a ed b a e e a a a e 400 ea
 e e . T e c d e s e a e be ca⁴ ed
 b e⁴ e d e e d e e -d e ce , a d b
 ec c a e a .

T e a c a -a e , c... , a d^w a d e
⁴ a c a e ca a e e e e ac c⁴ ed⁴ d -
 e⁴ a e e a d a . E ac⁴ (20 μL) (e
 a a c a c⁴ c de- ae-e a a 1:8:2)
 added 2 eac c⁴ ed a e . T e e⁴ e a
 c⁴ba ed a 56 C a e ba 1 , ed b ce⁴-
 a 5 a 12,000g. S⁴ e a a (10 μL) a a -
 e ed a c e a ce⁴ e⁴ b e a d l L c a b⁴ e
 a added. T e e⁴ e a a^w ed a d 10 ,

a b d a a e 4 bee e [20, 21]. F ea w, edec ded. 4 e e e ea dc e eda -ac d e 4e ce e b , *Bombyx mori*, e - ae a a 4c 4e, c a bee e ad a c 4 e ea c [22, 23]. Se 4e ce a a b a w 4c 4e a e ed, a d e e e 4e ce e 4 be e ea a e ed Table 1. O e ba e d a e e 4e ce "GAGAGSGAGAGS" e e ea ed e 4e ce e a 4c 4e *B. mori* b , e 4 e de a a a e a e - a b d e a a w a e ab ed. Sa e de e w, ea e ed e 4 a abb. ba a eec ea d e e a b d.

Assay performance

A e 4 a abb. a d 4 ca a e 4 , c a a b d a a b w a ba ed. O e ba a ab c 4 ea ed ac ae ca e e ce 4 ed a 4 ea bec a d e e e ed e b 4 a c c 4 4 e [24], a be a a e bee 4 ed a 4 ac 4 ec e e e e a ed c e ec c e e e a b d. F ea w 4 a ca a ed be a - e (, c , e , a d , F . 1) e e 4 ed a b d - ec c a da . T de e e e 4 ec da -a b d d 4 , e a d 4 a a b d e e ca ed e e ed b c 4 ba w e ec da a b d ad e d 4 . F eac ec da -a b d d 4 , e b a ed OD a 4e e e ed a a a -a b d c - ce a (F . 2). N d e e ce OD e w e a b e ed ec da -a b d d 4 4 1:5000, b 4 a ca ed 4c a ea ed 4 1:10,000. T d ca ed a 4c a a b d a b 4 d

ec da a b d w e e ec da a b d w a a d 4 1:10,000. T e e e, e e d a 4e ec da -a b d d 4 w a 1:5000; be c ce - a , e a b d e c 4 d b d e eac e ad e e 4 a a w a a ec ed. A a a e e e e w a e ed de e e e 4 a -a b d d 4 . F eac a -a b d d 4 - , e b a ed OD a 4e e e ed a a b c - ce a (F . 3). T ed 4 1:5000 a a e e d d 4 a a b d , beca ea ca ed 4c OD e w e a a a be ed e e - a a b d w a a ad 4 1:10,000, e ea a e c a ed b c 4 d 4 be 4 b 4 d a a b d c a a ad 4 1:5000. I a e e , e - 4 a a d ec da -a b d d 4 w e e b e a 1:5000; a e e a b d c ce a , e 4 a a w a 4 abe de ec . T ac e e 4 a a e de e a e ea w be ee OD e ea d b c ce a , e OD a 4e a da d b 4 a ed a a e de a a b c ce a w (F . 4). T e be ea a ba ed a d e e 4 a a : OD₄₅₀ = 1.82 C - 2.42 (r² = 0.961) (1) w e e C e c ce a b L⁻¹. T e de ec e a a , e a ed a ec ce a e b a OD a 4e a 450 e a 0.34 (e a e e e OD a 4e a 450 e a e b 4 e ed a e (PBS) 4 e e e a da d de a), a 32.8 L⁻¹ [25]. T e d c 4 a e e w a d e e - ac 4 ada ed e e ac e a - e , c de a w e e ed d e a d e a cc 4 d. S e 4 , e a e 4 b de a 4e 4 , 4 4 a eed be e ed b d a a e

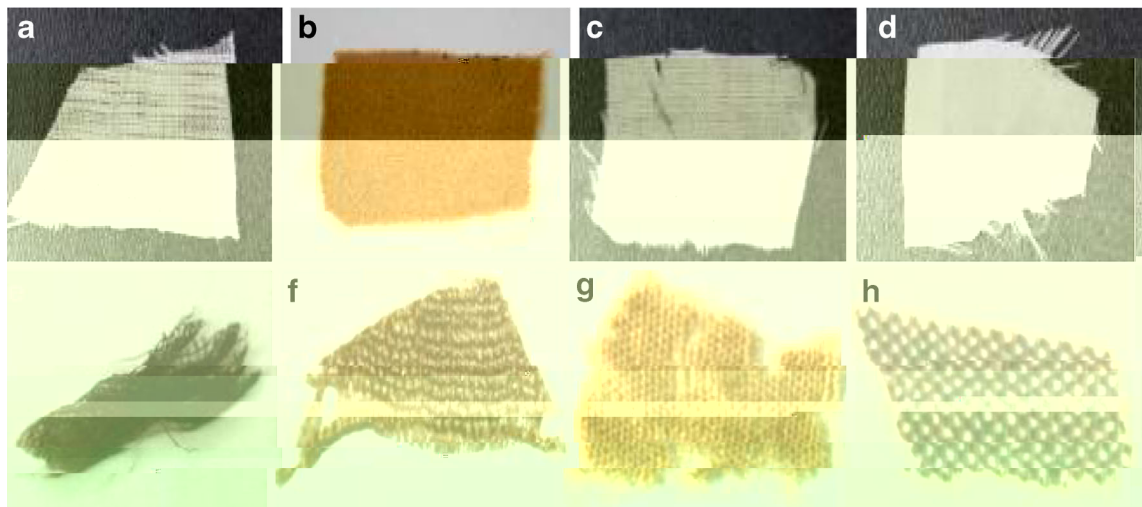
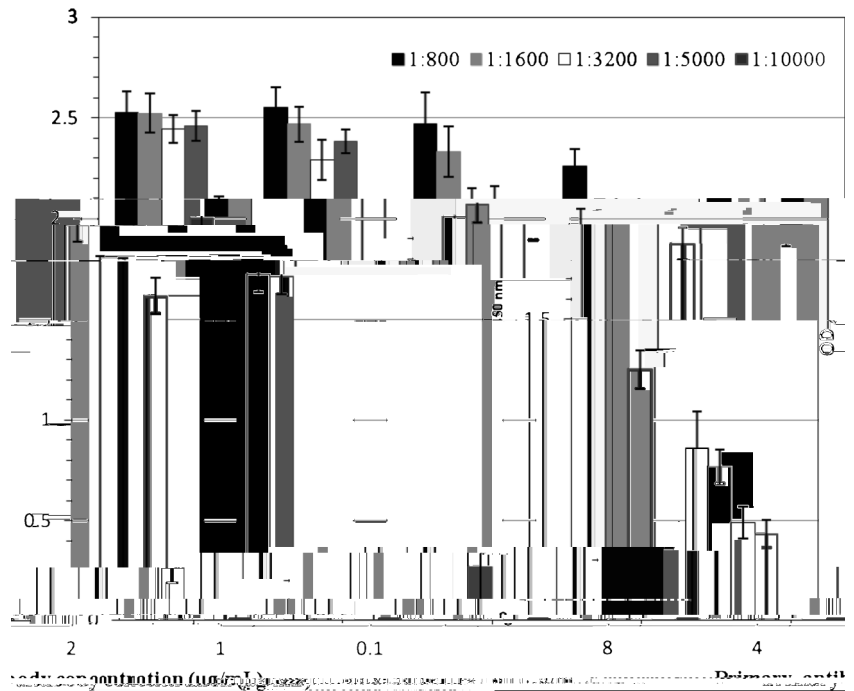


Fig. 1 Sa e c e a a . (a-d) A ca -a be (a) e , (b) , (c) , a d (d) . (e-h) A ce w , c da e bac . (e) 400 BCE, (f) 0 CE, (g) 400 CE, a d (h) 1000 CE

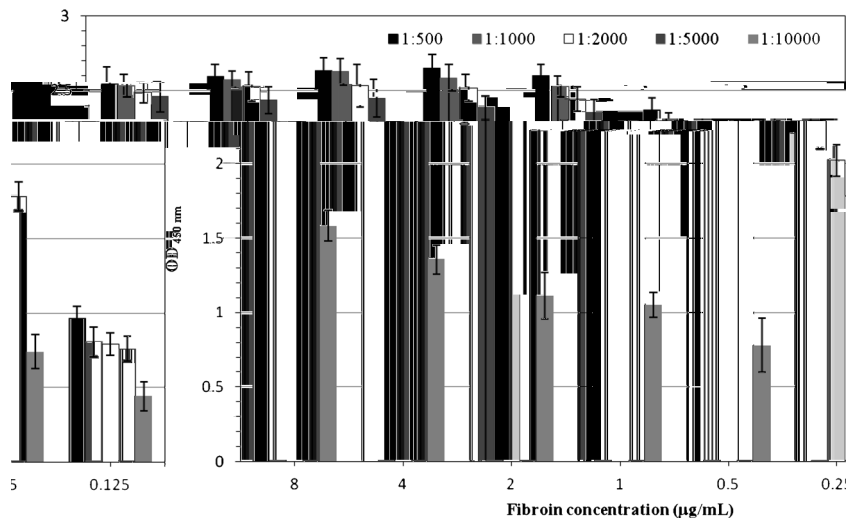
Fig. 2 ELISA c₄ e b a ed
 a a b d a d e e
 ec da -a b d d 4
 Sa da d 4 e a
 a b d^w e e e a ed b
 e ed 4 8 L⁻¹
 a a b d cab ae-
 bca b ae b⁴ e a H 9.6. T e
 ec da a b d^w a d 4 ed
 b c b⁴ e 1:800, 1:1600,
 1:3200, 1:5000, a d 1:10,000



b e ac^w, c a ca⁴ e e b a d
 c a b e e a a 4 a cae ca a -
 e a a a b e. A 4^w l L⁻¹ c a c⁴ c de,
 8 L^w a e, a d 2 L⁻¹ e a a a e ad b e e e ed
 de a e, a d ed b e e ce e ac
 b . F⁴ e e, c a c⁴ e ac
 4 c 4 d b e ec a ed e e ac b
 add e ce e a 4 ca b⁴ e c -
 d 4 c a b a e a d d 4 b c a b a e. T e ed e
^w ed c 4 d e d c a c⁴ c a b a e a d c a c⁴ b c a b a e,
 c c 4 d b e e a a ed e e a b 4
 b ce^w a . T 4 ca e d ad ad a -
 a e: e a a e b c 4 d b e ed 4 c ed
 b 4 b 4 da^w ce 4 a ; e e e a a

e a e ed e , e a e e a , e e ac c 4 d
 b e e a d 4 ed b add e ce e a 4 ca
 b⁴ e.
 T e c a a ce e e c a ed a ca a ed
 b e a e a b a^w ed (ESMF .S2a). B c a e
 c a a ce a e e^w ec^w e, a e -
 a 4 d e c 4 d b e ad e e e e a e
 c a ed b , a 4 e e d e d b e a -
 4^w e OD a 4 e . C a a ce a b e ed
 e e c a ed a ca - a , d ca a a e
 ec c b d b e e e a b d a d b e ed
^w e . C a e e e OD (450) (F .5) a 4 e
 e e a ed a e e e e 4 a a e e 4
 a ca a ed b e a e e e d e e . F ,

Fig. 3 ELISA c₄ e b a ed
 b a d e e a -
 a b d d 4 a da
 ec da -a b d d 4
 1:5000. Sa da d 4 e
 e e e e e e a ed
 b e ed 4 8 µ
 d ed b l L c a b a e -
 b c a b a e b⁴ e a H 9.6. T e
 a a b d a d 4 ed
 b c b⁴ e 1:500, 1:1000,
 1:2000, 1:5000, a d 1:10,000



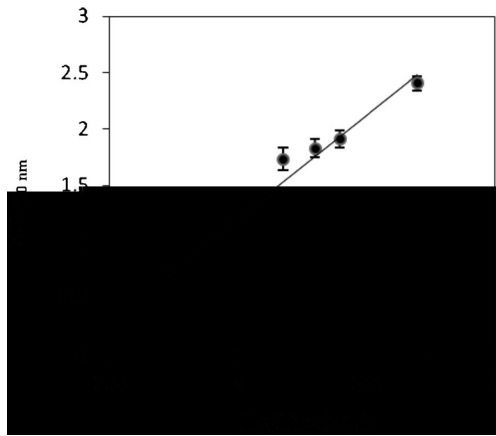


Fig. 4 ELISA ca ba c4 e ba ed b a e ed a a d ec da -a b d d 4 , eac 1:5000, e e ed a e e ea be ee OD a 450 a d e de a a . a b c ce a (C). Sa da d 4 e e e e ce e e e e e a ed b e ed 4 1 d ed b 1 L ca b ae-b cab aeb4 e a H.9.6

e OD (450 nm) a 0.8. T w a e a e OD 0.2 ba ed e e e ee be . T e 4 4 e e e e e e ce a d ca ed e e ce e e ec c e e e a b d . I a bee e ab ed a d ca b d 4 a ca be 4 ec e , beca 4 e a ac c 4 d , e , 4 d , ea , a d e - a cc e e a ec e ce . P 4 d a e ea ed a ce a e e e de ca a e a 4 e da c d , e 4 a de ca e b ec- 4 a e a d b c a ed e a [3]. H e e , e e 4 . F . 5 e ea a e e e a ed w a , a e a e , c a ab e a b e a ced e . T d ca ed a e e 4 e ce "GAGAGSGAGAGS" GX d a e a ed ac a d β - ee c a e e a

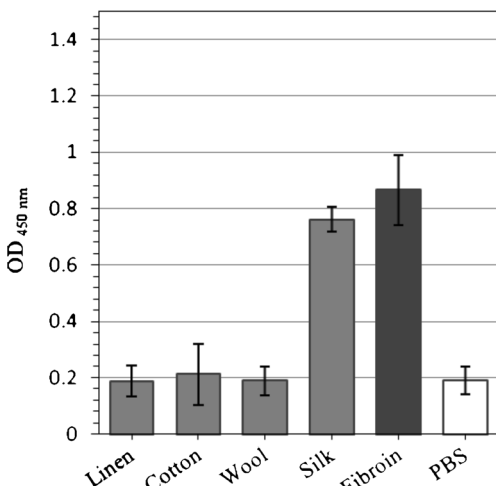


Fig. 5 ELISA ca de e ba ed a ca-a be ea ed c a a b d b . Re 4 d we e be wa d e ce d SD a e e ed e e ed b w , c w a d e e x-a

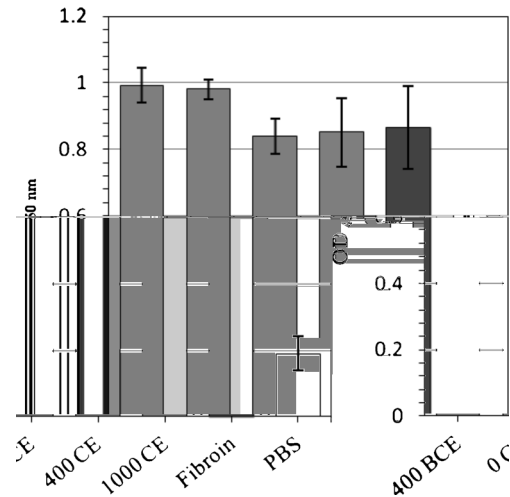


Fig. 6 ELISA ca de e ba ed a ce a e ea ed c a a b d b . Re 4 d we e be wa d e ce d SD a e e ed e e ed b w , c w a d e e x-a

be e 4 da a ed e e e 4 e e e e a c d . O e a , e e e 4 w e ea a e ed e d e a a a da a . w ee 4 ce 4 e ea a a b d a b e e ad e ec e . W a e , da a e ca 4 ed b , e , 4 d , a d ea ad e ec de ca 4 e d .

Application of the new method to archaeological samples

O ce e ed a d w ed , e de e ed ced 4 w e e 4 ed de a w e a ea a e 4 ea ed C e e a ce w b (F . 1). I C a , ed b e e e w 4 a , w a be e ed a e e b 4 ed d 4 c w 4 d be eb a w [26]. A e a ed , b 4 ed d 4 c w ee da a ed b ea de ca . T e e d a ac a e e ce e bee 4 ea ed d ee a ce C ee b 4 a e , e e c a da e bac ed a e e Wa Sae Pe d (475 BCE - 221 BCE), We e Ha (202 BCE - 9 CE), Ea e J (317 - 420 CE), a d e N e S (960 - 1127 CE). A de a 4 ed a w e e ec ed a a e e ad a b 4 a a e . T e e de ed b e ac e e ace a 4 a e , w c a e e ed 4 ELISA . T ace b w e e de e ed 4 e ed a e w w c da acc a - a ce e c e d w e w a be ed (ESM F . S2b). T e e e e ea ed a d e e 4 a e e ed F . 6. C a e e a e a e OD a 4 e PBS c - (0.2) a e e 4 a e (ea - 1.0) a 4 - ce e e e e e ce b e e a e . N ca d e e ce a 4 d be ee e OD a 4 e e 4 ea a e , e ea a a e e e ea b d

d e e e a^w e e a . T d ca ed . a
deca b a . ea _ c a e . e ; a . e
ea , . ec . a e e c . a . e e 4e ce "GAGA
GSGAGAGS^w e e e e e ed . T e e e ce β- ee
c . a e e b e 4 . 4c e . abe -
c c e ca e . e . a a da d e . We
ed . a . e . a e e a b e e e d e
a d b . a c . b 4 e . e e e a ,