

**Antihepca™ - Heparin resistant recalcifying solution  
#X9107-HX (For Research Use Only)**

**Intended Use**

Antihepca™ is to be used as a heparin neutralising agent in the recalcifying step of APTT, surface activated clotting tests (SACT, KCT) and other tests.

It is dyed light blue to avoid confusion with regular 0.025M calcium chloride. It is currently available in 5 x 10ml vials. The Antihepca™ is stable for at least 5 years stored 2-8°C.

**Method Principle**

Test plasmas giving prolonged APTT, SACT or KCT results with regular 0.025M calcium chloride may contain heparin or heparin-like substances which may be responsible for the test prolongation. Such plasmas will usually give shorter results when the APTT, SACT or KCT is repeated using Antihepca™. The concentration of anti-heparin agent is enough to neutralize up to 0.5u/ml heparin in most cases but is not so high that it affects APTT results prolonged by other abnormalities or defects.

Polybrene, protamine or similar cationic heparin neutralizing agents should not be added to APTT reagents or test plasmas because they can interfere with the contact activation by negatively charged surfaces occurring in the APTT preincubation stage.

**Test Procedure**

APTT and SACT tests are usually carried out by preincubating 0.1ml of test plasma with 0.1ml of a contact activating (APTT) reagent for 3-5minutes at 37°C, then adding 0.1ml of 0.025M calcium chloride and timing to a clotting endpoint. Proportionally smaller volumes are often used in automated clotting instruments.

To check for heparin in a test sample with a prolonged APTT the test should be repeated but using Antihepca in place of the regular 0.025M CaCl<sub>2</sub>.

**Results**

It can be seen from the results overleaf that 0.4u/ml of heparin in normal plasma (PNP) significantly prolonged APTT results depending on the APTT reagent used. A wide range of abnormal plasmas spiked with 0.2u/ml of heparin mostly gave even longer APTT results. Substituting Antihepca™ for regular 0.025M CaCl<sub>2</sub> reversed the effect of heparin so that the APTT results reverted to closely resemble those obtained without heparin.

This works with abnormal plasmas containing DOACs, lupus anticoagulants (LAC), D-dimer and factor deficient plasmas. Note that results obtained with different APTT reagents may vary slightly with this product (as shown) and thus should be interpreted with caution. In particular low molecular weight (LMW) heparins may be corrected to varying degrees.

The tables overleaf shows typical results obtained on various in vitro-spiked test plasmas using typical APTT reagents and 3 minute preincubation times on a ST4 instrument.

PNP= Pooled normal plasma. PBL=Precision BioLogic. Factor deficient plasmas from HBM (Hyphen BioMed).

**Significance**

Antihepca™ may be particularly useful in patients after cardiopulmonary bypass when heparin may not have been reversed adequately. It can also identify plasmas containing heparin accidentally or unexpectedly. It may be useful in APTT-based factor assays to give more reliable results despite the presence of heparin in a test sample. Note that the Antihepca™ should be diluted 1:10 with regular 0.025M CaCl<sub>2</sub> if used in factor assays (contact Haematex for more information).

Polybrene and other positively charged polymers have long been used to neutralize heparin in plasmas. However positively charged polymers bind to the anionic contact activating agents used in 2 stage clotting tests such as the APTTs and prolong such tests in an unpredictable manner. Thus heparin neutralization with polybrene added to plasma or APTT reagents is unreliable.

Cationic anti-heparin agents added to the recalcifying solution obviously do not affect the preceding contact activation stage but do neutralize heparin if it is present in a test plasma sample. Thus APTT results which tend to be shorter when Antihepca™ is used rather than plain calcium chloride indicate that heparin or a heparin-like agent\* may be present in the test plasma. It is apparent from these results that heparin does not interfere with contact activation.

*\*It has been reported that high levels of FDP/Dimer (>10µg/ml) may mimic heparin.*

**Cautions**

Contains azide (<0.02%).

**Table 1 shows results obtained on test plasmas with various LAC APTT resistant reagents used with 0.025M calcium chloride or Antihepca™**

Test plasma		Intrinsin LR (HTX)		Cephascreen (Stago)		Triniclot S (Stago)		Actin FS (Siemens)		Hemosil SynthFax (IL)	
		CaCl <sub>2</sub>	Antihepca	CaCl <sub>2</sub>	Antihepca	CaCl <sub>2</sub>	Antihepca	CaCl <sub>2</sub>	Antihepca	CaCl <sub>2</sub>	Antihepca
Normal	PNP.HTX	30.7	30.1	33.6	31.3	33.9	35.0	29.8	29.3	24.4	27.8
Anticoags	aa + Heparin 0.4IU/ml	114	34.5	71.8	35.2	83.0	40.9	70.3	30.6	51.7	30.4
	PNP + Clexane 0.2IU/ml	51.2	36.0	46.4	32.6	58.0	41.3	48.1	30.4	38.6	28.0
	Rivaroxaban 400ng.ml	52.2	52.1	50.8	52.8	58.4	58.0	54.3	52.1	38.2	42.9
	aa + Heparin 0.2IU/ml	131	52.4	92.8	53.3	112	70.0	115	49.1	68.5	46.3
	Dabigatran 200ng.ml	60.2	60.8	60.0	59.5	79.8	74.7	58.6	58.2	45.5	57.3
	aa + Heparin 0.2IU/ml	119	61.9	86.3	60.7	125	81.8	94.3	52.3	68.1	50.8
	Adsorbd Plas.+ 30%NP	77.8	76.3	85.8	86.4	100	91.3	92.8	83.0	86.9	88.5
	aa + Heparin 0.2IU/ml	>200	77.8	>200	95.3	>200	120	>200	94.0	>200	116
Defic.	20% Factor IX	40.8	39.2	38.1	38.9	44.2	43.7	41.0	34.6	29.5	31.9
	aa + Heparin 0.2IU/ml	75.3	42.5	51.7	42.0	58.7	46.1	51.4	32.0	39.5	33.7
	20% Factor XI	43.8	42.6	44.0	44.8	50.8	44.0	42.6	43.6	38.1	40.4
	aa + Heparin 0.2IU/ml	85.9	46.5	64.0	45.7	77.0	50.0	73.3	37.1	59.1	41.1
Other	D-Dimer (2µg/ml)	77.9	74.0	67.1	66.2	77.6	70.2	75.1	66.1	47.9	53.0
	aa + Heparin 0.2IU/ml	>200	77.7	102	69.8	130	75.9	158	67.7	71.0	56.5
	PNP + Polybrene 0.001%	76.3	85.1	35.3	35.3	42.9	47.0	68.3	88.0	>200	>200
LAC	LAC pos.#168	31.3	30.7	37.0	37.1	55.9	55.3	29.0	26.1	25.3	28.0
	aa + Heparin 0.2IU/ml	51.1	32.6	49.2	39.5	82.8	62.8	40.0	26.5	31.4	27.4
	Weak LAC (PBL)	36.1	34.4	38.9	36.2	54.2	50.1	32.3	29.5	29.0	30.9
	LAC pos.(PBL)	35.5	34.5	42.1	40.1	62.5	59.6	30.8	28.5	30.5	32.7

**Table 2 shows results obtained similarly using several LAC sensitive APTT reagents.**

Test plasma		Intrinsin LS (HTX)		Triniclot HS (Stago)		Hemosil Synthasil (IL)		Actin FSL (Siemens)		Hemosil SP (IL)	
		CaCl <sub>2</sub>	Antihepca	CaCl <sub>2</sub>	Antihepca	CaCl <sub>2</sub>	Antihepca	CaCl <sub>2</sub>	Antihepca	CaCl <sub>2</sub>	Antihepca
Normal	PNP.HTX	35.3	35.4	32.6	32.7	34.5	38.4	29.3	28.2	32.6	36.9
Anticoags	aa + Heparin 0.4IU/ml	77.1	36.2	103	38.7	109	46.7	69.0	34.7	83.5	42.4
	PNP + Clexane 0.2IU/ml	58.6	38.6	55.3	38.9	57.3	48.2	46.8	37.6	56.6	43.6
	Rivaroxaban 400ng.ml	53.5	54.5	50.5	53.9	58.0	64.2	48.9	53.8	58.0	61.0
	aa + Heparin 0.2IU/ml	97.8	49.0	116.0	57.3	119	65.8	83.1	49.8	121	65.1
	Dabigatran 200ng.ml	71.4	73.9	61.9	63.9	65.6	78.7	56.4	56.8	68.4	68.8
	aa + Heparin 0.2IU/ml	109	64.5	119	67.0	130	81.3	86.0	58.5	113	73.7
	Adsorbd Plas.+ 30%NP	64.0	56.5	70.6	77.9	63.7	76.6	75.5	75.1	76.2	75.8
	aa + Heparin 0.2IU/ml	156	60.5	>200	85.8	150	80.5	184	77.7	>200	82.0
Defic.	20% Factor IX	42.5	40.9	44.6	43.6	54.8	58.4	37.2	37.5	45.1	43.7
	aa + Heparin 0.2IU/ml	61.8	42.9	76.8	48.9	125	71.9	51.5	39.9	73.1	48.0
	20% Factor XI	44.8	38.8	46.4	44.8	54.5	58.1	39.4	40.5	44.6	44.3
	aa + Heparin 0.2IU/ml	68.1	44.1	91.0	50.5	118	75.1	58.6	43.1	78.8	48.6
Other	D-Dimer (2µg/ml)	76.0	73.7	72.7	70.9	82.5	82.6	81.7	82.3	73.7	72.9
	aa + Heparin 0.2IU/ml	158	72.1	172	81.0	186	90.3	181	88.1	157	80.5
	PNP + Polybrene 0.001%	153	>200	37.7	36.8	>200	>200	40.7	42.5	37.8	37.0
LAC	LAC pos.#168	105	93.2	53.8	54.7	52.9	63.8	32.0	36.0	59.3	63.5
	aa + Heparin 0.2IU/ml	>200	93.5	95.7	59.8	102	72.9	49.1	40.3	89.3	66.4
	Weak LAC (PBL)	94.8	93.8	48.5	47.5	69.2	74.4	33.8	34.2	77.4	74.0
	LAC pos.(PBL)	108	105	55.9	55.7	71.6	79.3	38.4	41.5	104	99.9

**SUMMARY:** In every case APTT tests using Antihepca™ instead of 0.025M CaCl<sub>2</sub> gave significantly shorter results only if heparin was present in the test plasma. The polybrene in Antihepca™ did not affect most of the APTT results significantly if heparin was not present. (Except for some results with Synthasil and Actin FSL).