

V. SIMPULAN DAN SARAN

A. Simpulan

Berdasarkan penelitian yang telah dilakukan, didapatkan simpulan sebagai berikut.

1. Batas aman penggunaan konsentrasi PEG 4000 yang tidak menyebabkan hiperagregasi pada sel darah merah ayam yaitu 12% (w/v) dan pada mencit (BALB/c) yaitu 7% (w/v)
2. Penambahan konsentrasi PEG dalam batas aman (5% w/v) tidak memberikan pengaruh terhadap uji hemaglutinasi virus ND terhadap sel darah merah ayam.
3. Desain kombinasi perlakuan yang paling memberikan pengaruh terhadap aktivitas hemaglutinasi sel darah merah mencit (BALB/c) dalam uji hemaglutinasi virus SARS-CoV-2 yaitu kombinasi praperlakuan *neuraminidase* 41 U/mL terhadap virus SARS-CoV-2, penambahan PEG (4000) konsentrasi akhir 33% (v/v), waktu inkubasi selama 60 menit dengan nilai titer tertinggi yaitu $2^{7.58}$ HAU pada temperatur inkubasi 25°C dan 2^8 HAU pada temperatur inkubasi 37°C.

B. Saran

Berdasarkan penelitian yang telah dilakukan, dapat diberikan saran sebagai berikut.

1. Perlu dilakukan penelitian dengan metode serupa yang dilakukan oleh peneliti lain sebagai bentuk validasi metode analisis parameter akurasi, presisi dan spesifitas.
2. Perlu dilakukan penelitian dengan metode yang serupa terhadap sampel supernatan isolat virus SARS-CoV-2 inaktif yang telah dipurifikasi menjadi antigen murni.
3. Perlu dilakukan penelitian lebih lanjut terhadap pengujian kualitas aglutinasi secara mikroskopis.

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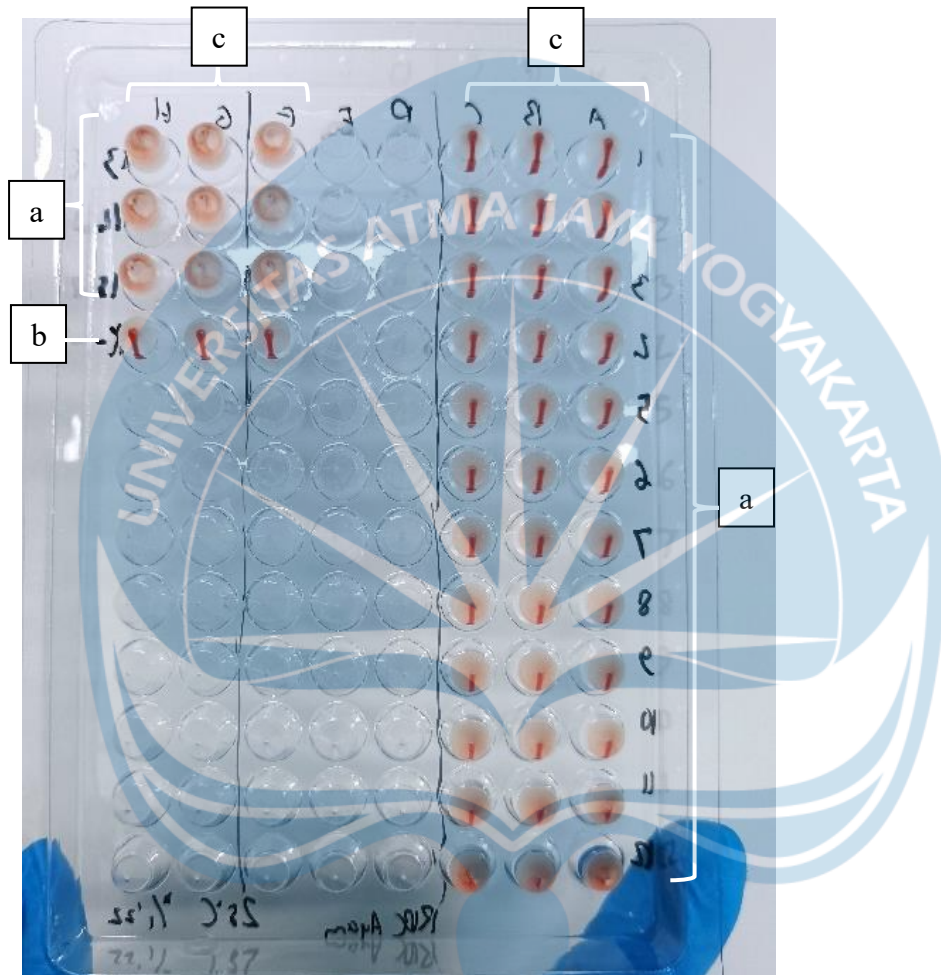
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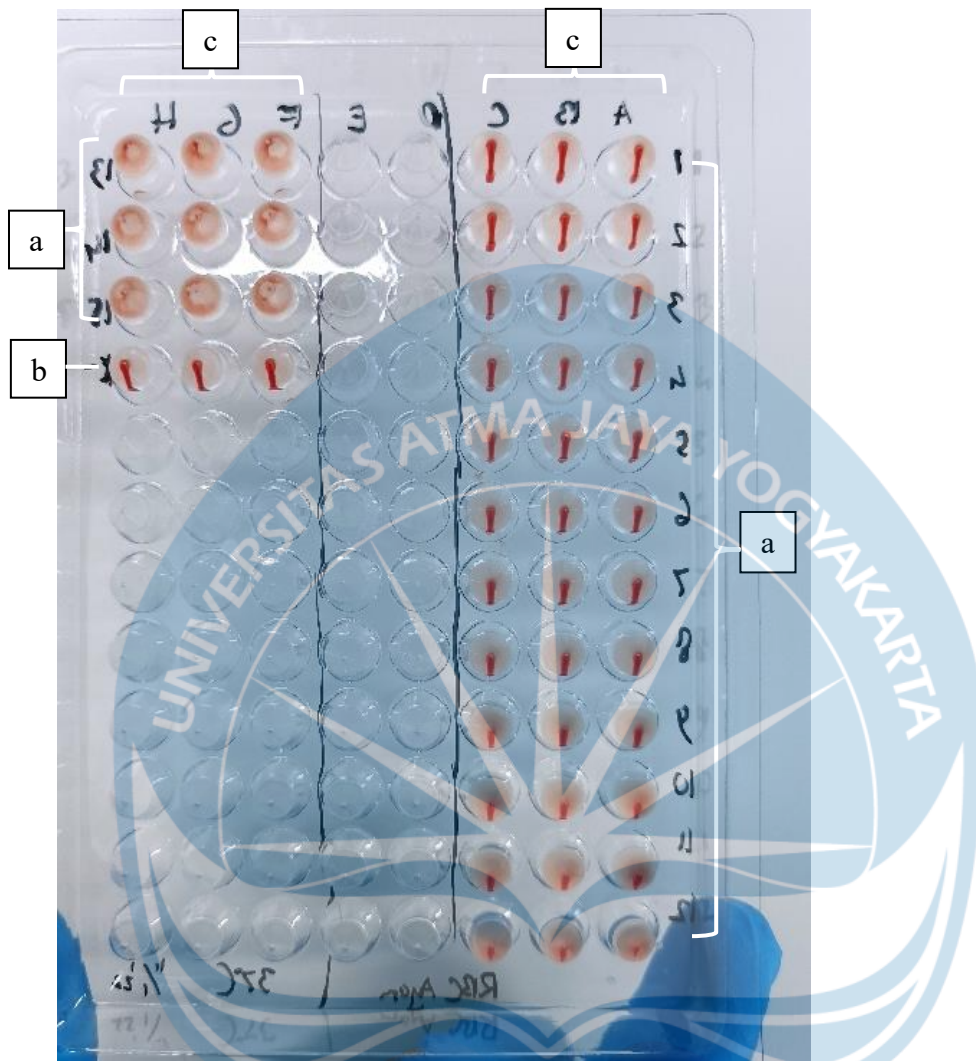


LAMPIRAN

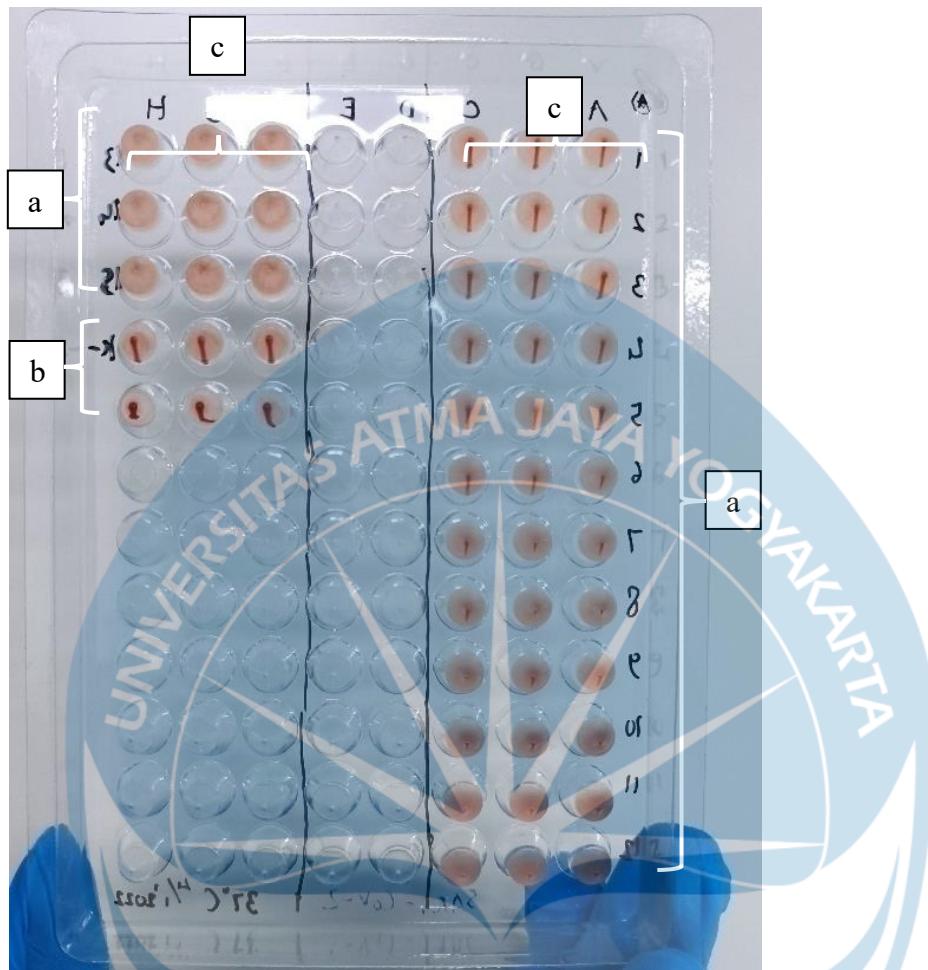
A. Lampiran Gambar



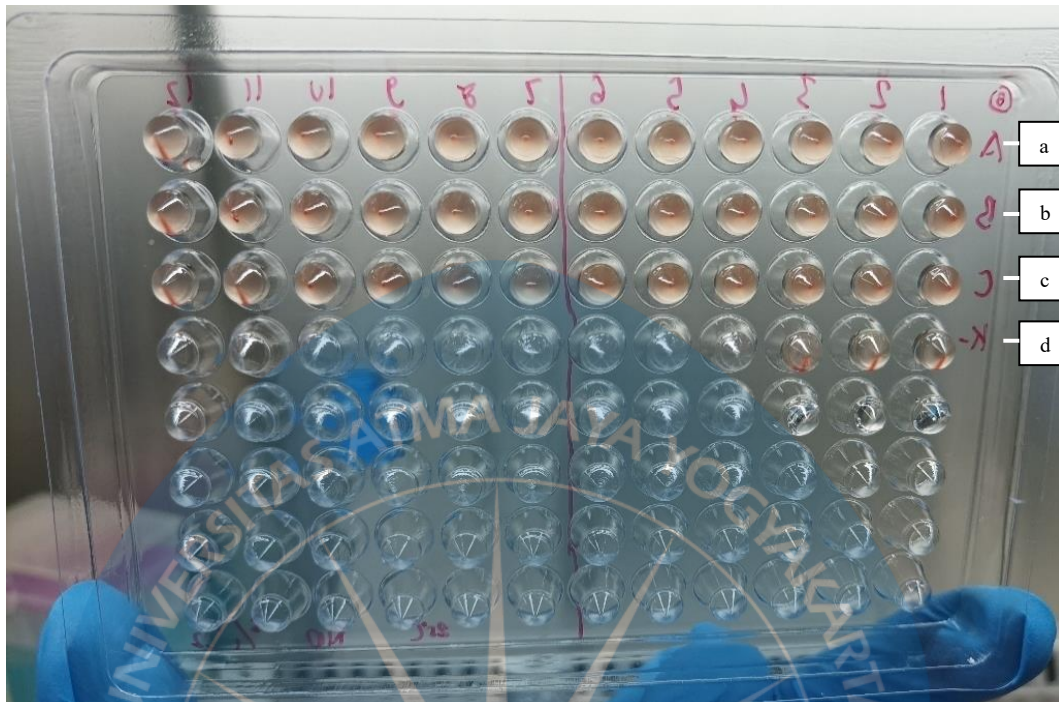
Gambar 14. Hasil uji hemaglutinasi PEG (1-15%) terhadap sel darah ayam 1% pada temperatur inkubasi 25°C selama 60 menit (a) Dengan variasi konsentrasi larutan stok PEG 1-15% (w/v); (b) Kontrol negatif tanpa PEG; (c) Pengujian dilakukan triplo



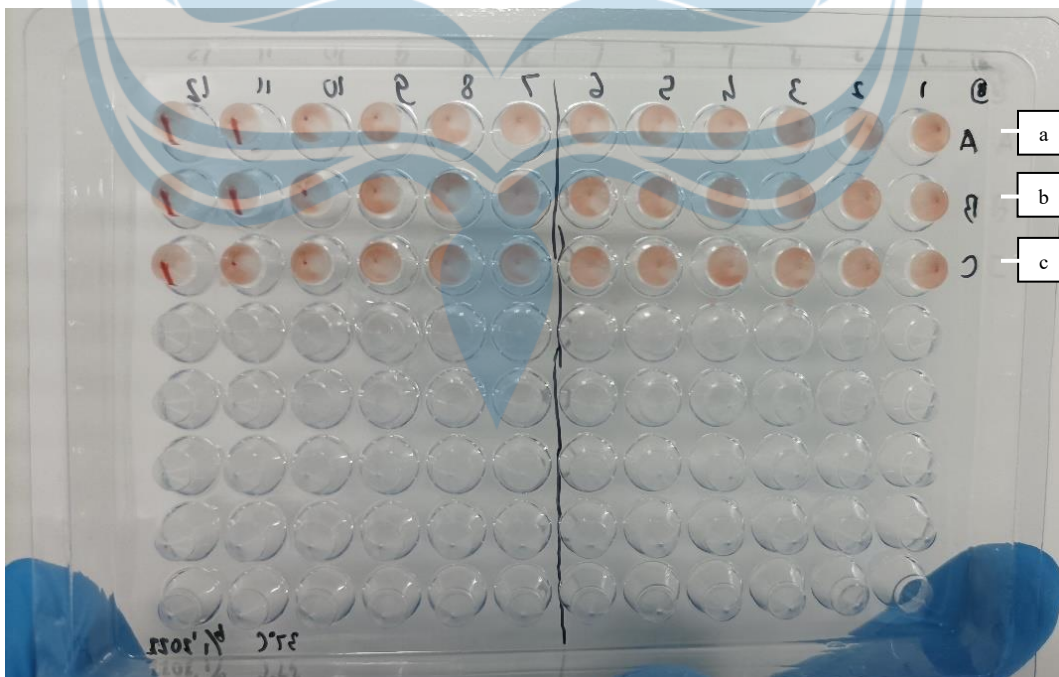
Gambar 15. Hasil uji hemaglutinasi PEG (1-15%) terhadap sel darah ayam 1% pada temperatur inkubasi 37°C selama 60 menit (a) Dengan variasi konsentrasi larutan stok PEG 1-15% (w/v); (b) Kontrol negatif tanpa PEG; (c) Pengujian dilakukan triplo



Gambar 16. Hasil uji hemaglutinasi PEG (1-15%) terhadap sel darah merah mencit (BALB/c) 1% pada temperatur inkubasi 37°C selama 60 menit (a) Dengan variasi konsentrasi larutan stok PEG 1-15% (w/v); (b) Kontrol negatif tanpa PEG; (c) Pengujian dilakukan triplo



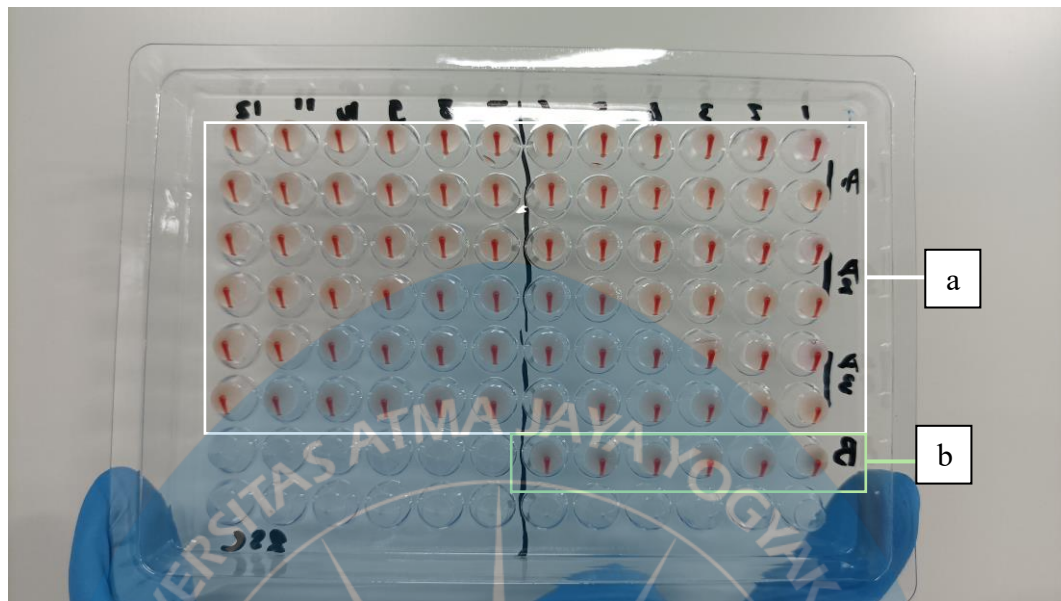
Gambar 17. Hasil uji hemaglutinasi virus ND terhadap sel darah merah ayam 1% pada temperatur inkubasi 25°C selama 30 menit (a) Ulangan ke-1; (b) Ulangan ke-2; (c) Ulangan ke-3; (d) Kontrol negatif tanpa virus ND



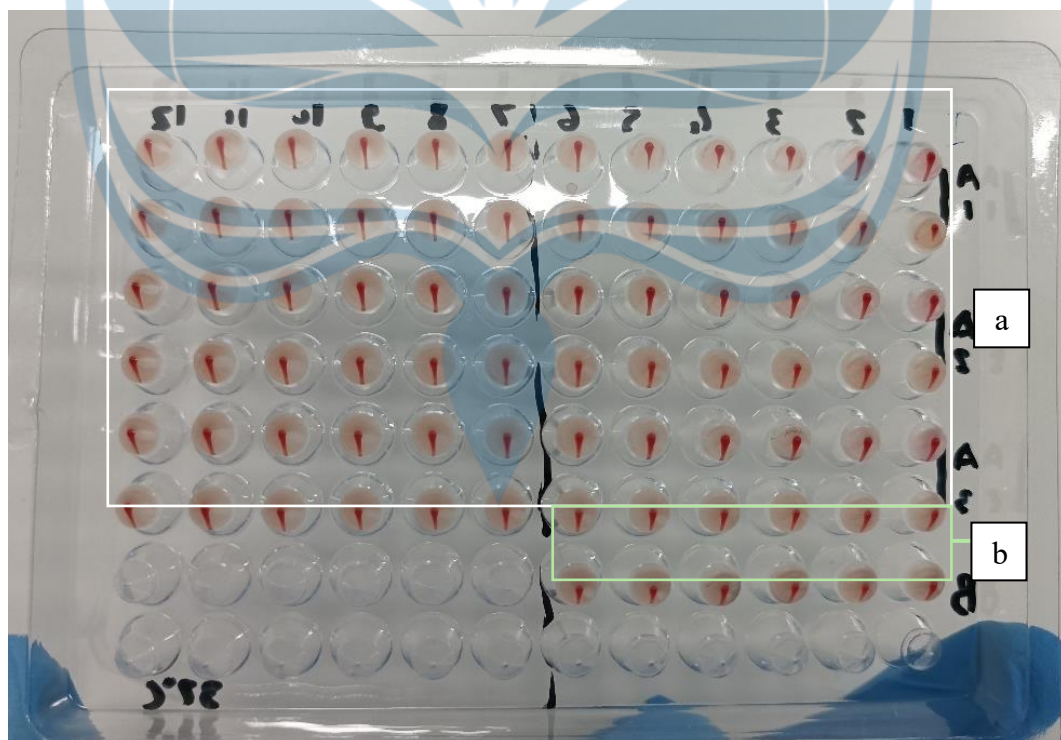
Gambar 18. Hasil uji hemaglutinasi virus ND terhadap sel darah merah Ayam 1% pada temperatur inkubasi 37°C selama 30 menit



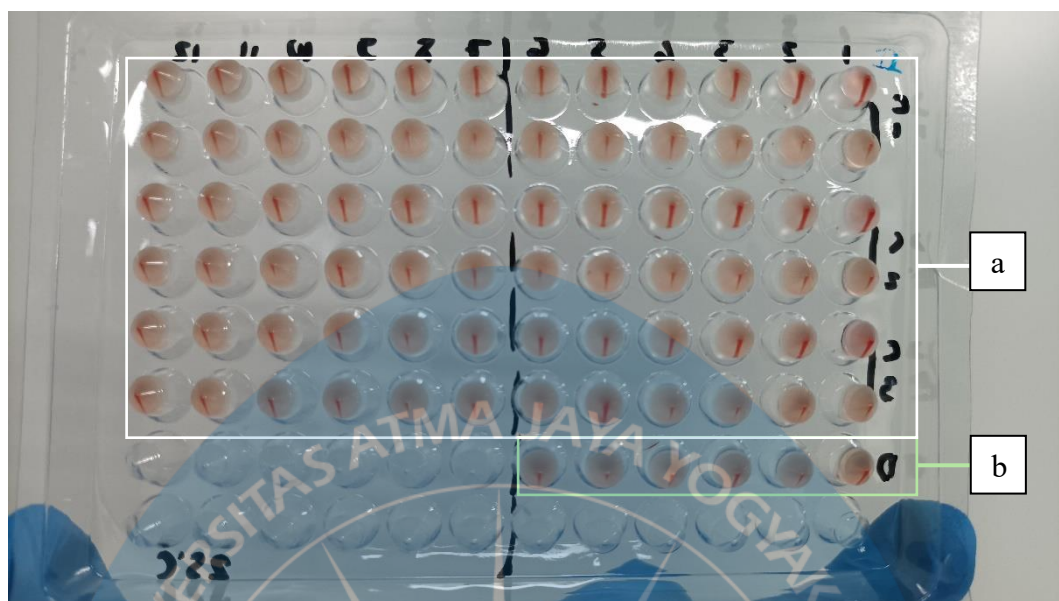
Gambar 19. Hasil uji hemaglutinasi virus ND dengan penambahan PEG 5% terhadap sel darah merah ayam 1% pada temperatur inkubasi 37°C selama 30 menit (a) Ulangan ke-1; (b) Ulangan ke-2; (c) Ulangan ke-3



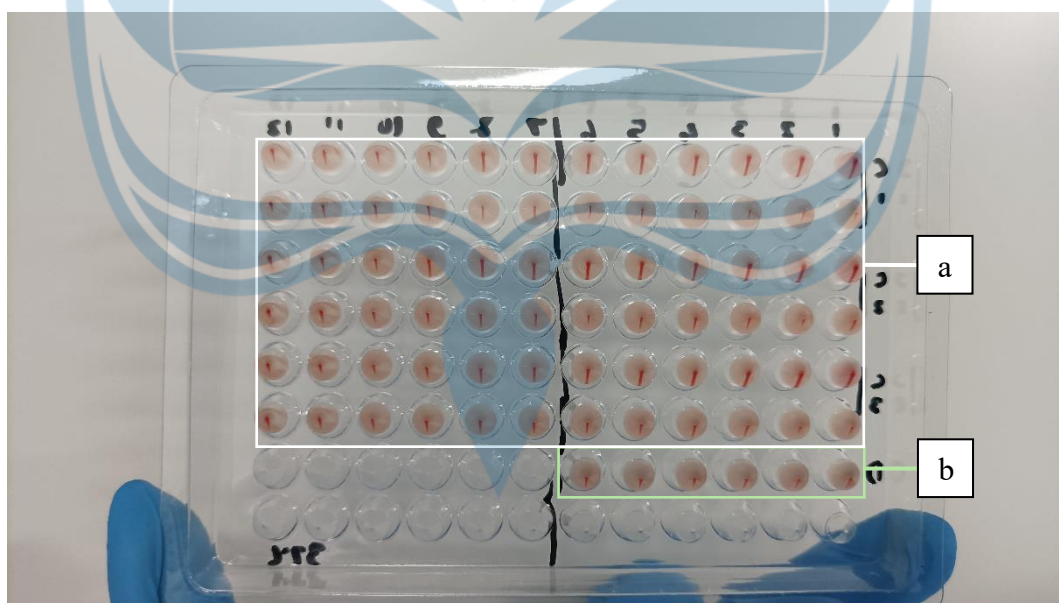
Gambar 20. Hasil uji hemaglutinasi pada temperatur inkubasi 25°C selama 60 menit (a) HA Virus SARS-CoV-2 terhadap sel darah merah mencit (BALB/c) 1% dengan nilai titer 0 HAU; (b) Kontrol negatif (HA tanpa virus)



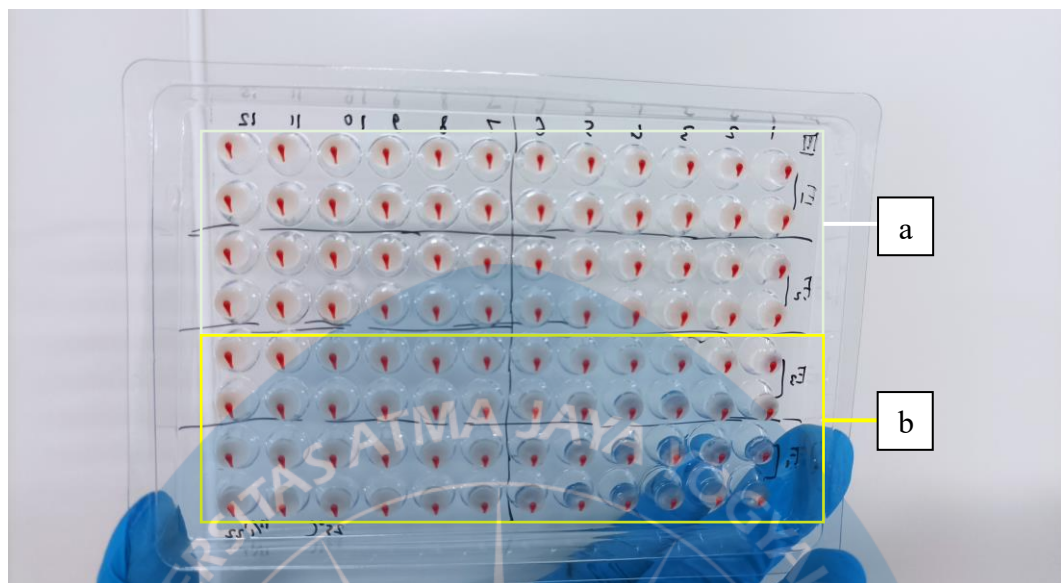
Gambar 21. Hasil uji hemaglutinasi pada temperatur inkubasi 37°C selama 60 menit (a) HA virus SARS-CoV-2 terhadap sel darah merah mencit (BALB/c) 1% dengan nilai titer 0 HAU; (b) Kontrol negatif (HA tanpa virus)



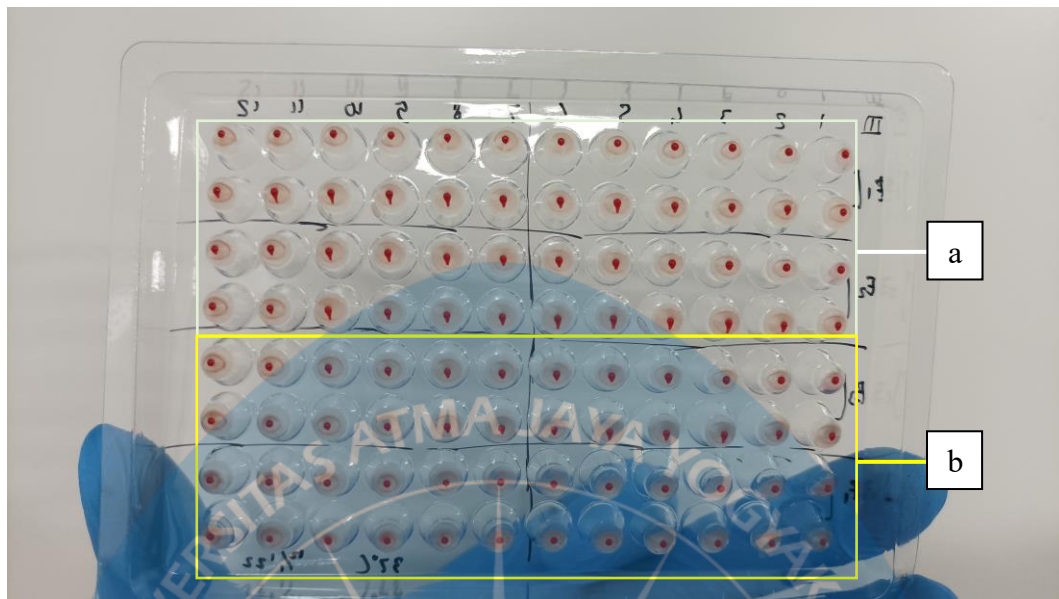
Gambar 22. Hasil uji hemaglutinasi dengan penambahan PEG 33,33% pada temperatur inkubasi 25°C selama 60 menit (a) HA virus SARS-CoV-2 terhadap sel darah merah mencit (BALB/c) 1% dengan nilai titer 0 HAU; (b) Kontrol negatif (HA tanpa virus)



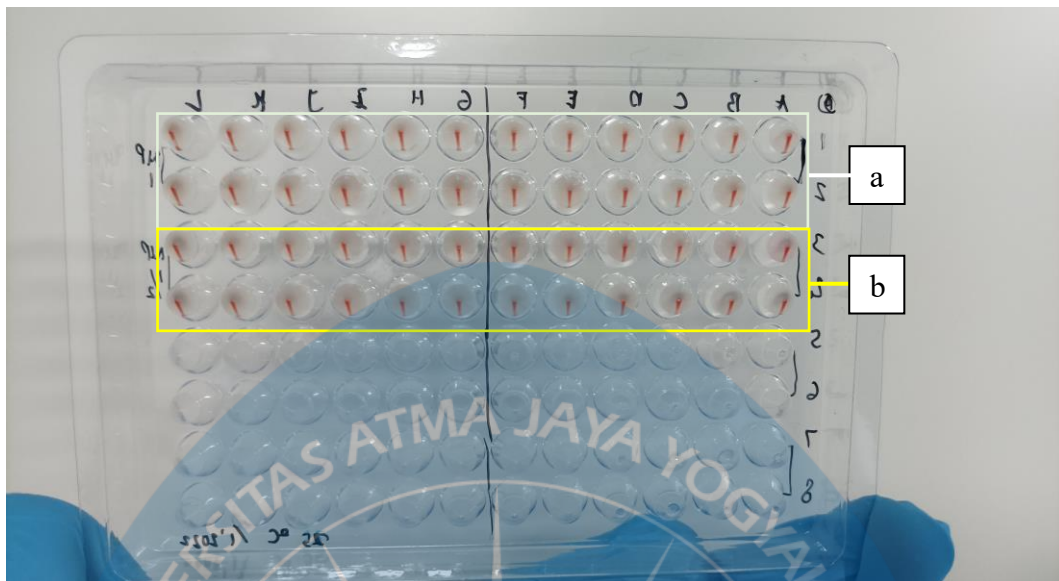
Gambar 23. Hasil uji hemaglutinasi dengan penambahan PEG 33,33% pada temperatur inkubasi 37°C selama 60 menit (a) HA virus SARS-CoV-2 terhadap sel darah merah mencit (BALB/c) 1% dengan nilai titer 0 HAU; (b) Kontrol negatif (HA tanpa virus)



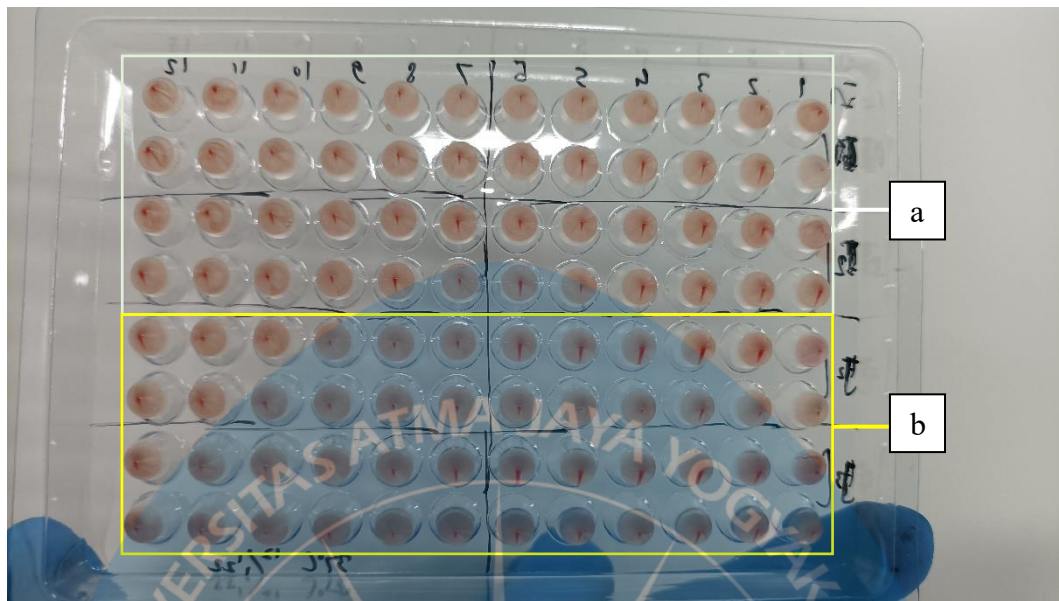
Gambar 24. Hasil uji hemaglutinasi dengan praperlakuan *neuraminidase* pada temperatur inkubasi 25°C selama 60 menit (a) HA virus SARS-CoV-2 praperlakuan *neuraminidase* 5 U/mL terhadap sel darah merah mencit (BALB/c) 1% dengan nilai titer 0 HAU; (b) HA virus SARS-CoV-2 praperlakuan *neuraminidase* 10 U/mL terhadap sel darah merah mencit (BALB/c) 1% dengan nilai titer 0 HAU



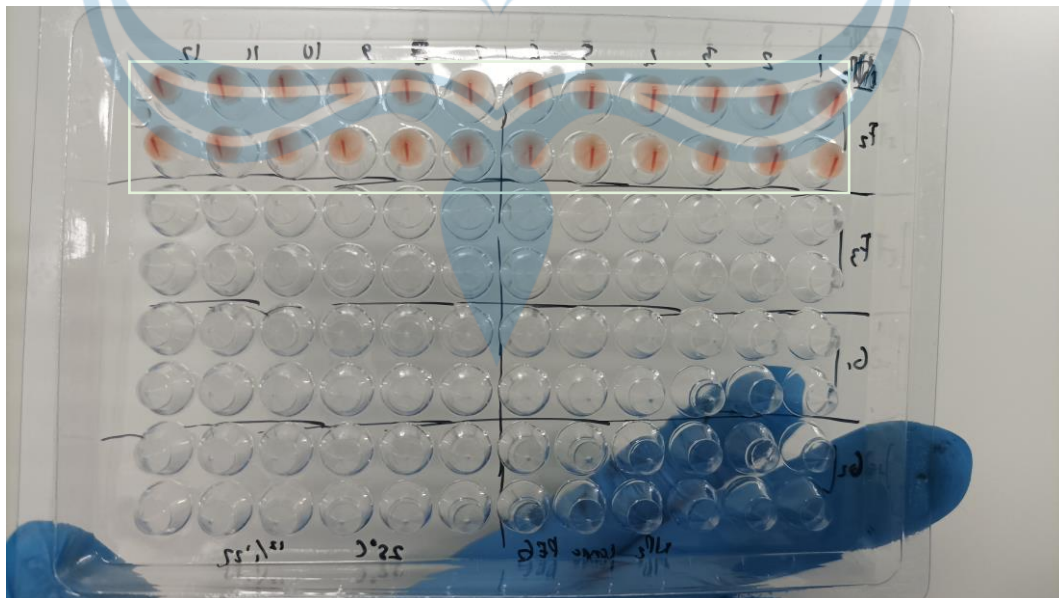
Gambar 25. Hasil uji hemaglutinasi dengan praperlakuan *neuraminidase* pada temperatur inkubasi 37°C selama 60 menit (a) HA virus SARS-CoV-2 praperlakuan *neuraminidase* 5 U/mL terhadap sel darah merah mencit (BALB/c) 1% dengan nilai titer 0 HAU; (b) HA virus SARS-CoV-2 praperlakuan *neuraminidase* 10 U/mL terhadap sel darah merah mencit (BALB/c) 1% dengan nilai titer 0 HAU



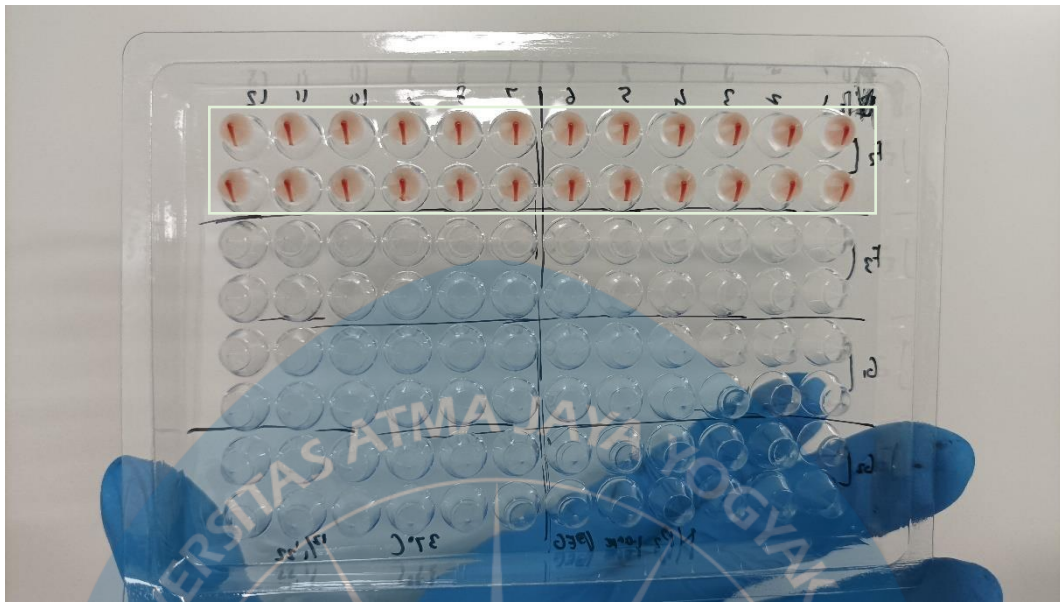
Gambar 26. Hasil uji hemaglutinasi dengan praperlakuan *neuraminidase* dan penambahan PEG 33,33% pada temperatur inkubasi 25°C selama 60 menit (a) HA virus SARS-CoV-2 praperlakuan *neuraminidase* 10 U/mL terhadap sel darah merah mencit (BALB/c) 1% dengan nilai titer 0 HAU; (b) HA virus SARS-CoV-2 praperlakuan *neuraminidase* 5 U/mL terhadap sel darah merah mencit (BALB/c) 1% dengan nilai titer 0 HAU



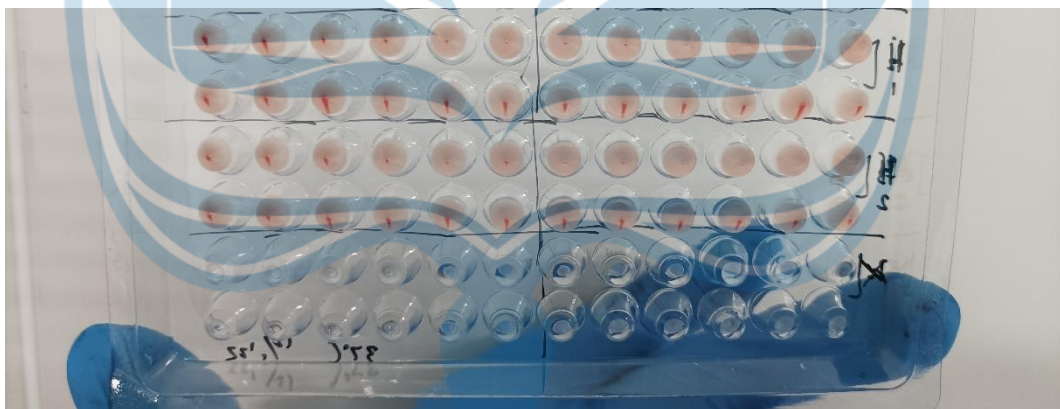
Gambar 27. Hasil uji hemaglutinasi dengan praperlakuan *neuraminidase* dan penambahan PEG 33,33% pada temperatur inkubasi 37°C selama 60 menit (a) HA virus SARS-CoV-2 praperlakuan *neuraminidase* 10 U/mL terhadap sel darah merah mencit (BALB/c) 1% dengan nilai titer 0 HAU; (b) HA virus SARS-CoV-2 praperlakuan *neuraminidase* 5 U/mL terhadap sel darah merah mencit (BALB/c) 1% dengan nilai titer 0 HAU



Gambar 28. Hasil uji hemaglutinasi virus SARS-CoV-2 dengan praperlakuan *neuraminidase* 41 U/mL terhadap sel darah merah mencit (BALB/c) 1% pada temperatur inkubasi 25°C selama 60 menit dengan nilai titer 0 HAU



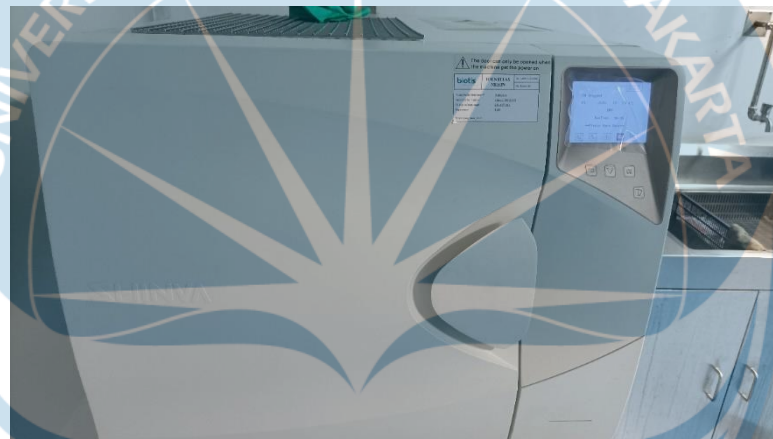
Gambar 29. Hasil uji hemaglutinasi virus SARS-CoV-2 dengan praperlakuan *neuraminidase* 41 U/mL terhadap sel darah merah mencit (BALB/c) 1% pada temperatur inkubasi 37°C selama 60 menit dengan nilai titer 0HAU



Gambar 30. Hasil uji hemaglutinasi virus SARS-CoV-2 dengan praperlakuan *neuraminidase* 41 U/mL dan penambahan PEG 33,33% terhadap sel darah merah mencit (BALB/c) 1% pada temperatur inkubasi 37°C selama 60 menit



Gambar 31. Prosedur Berpakaian dan Penggunaan Alat Pelindung Diri (APD)



Gambar 32. Autoklaf 2017L330 (Shinva)



Gambar 33. Alat Sterilisasi Panas Kering (Shinva)



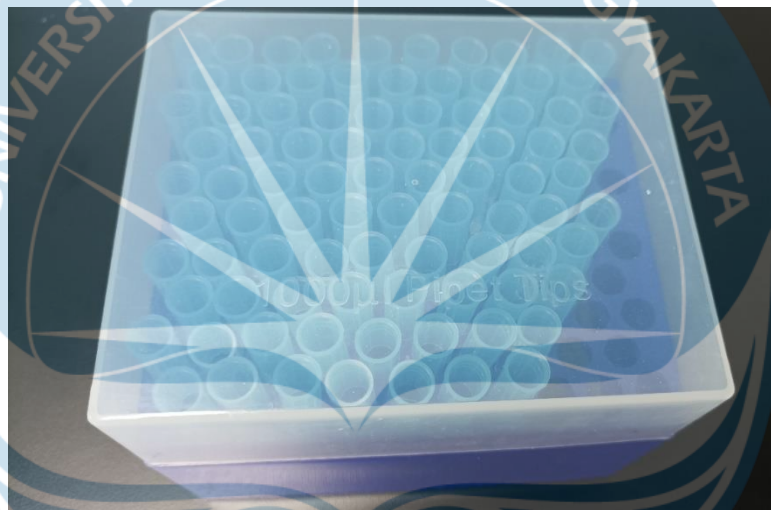
Gambar 34. Deep Freezer -23°C (Haier Biomedical 92/262 Series)



Gambar 35. Lemari Es -8°C (Haier HYC-310S)



Gambar 36. Biosafety Cabinet II/A2/JT18030725 (Airtech)



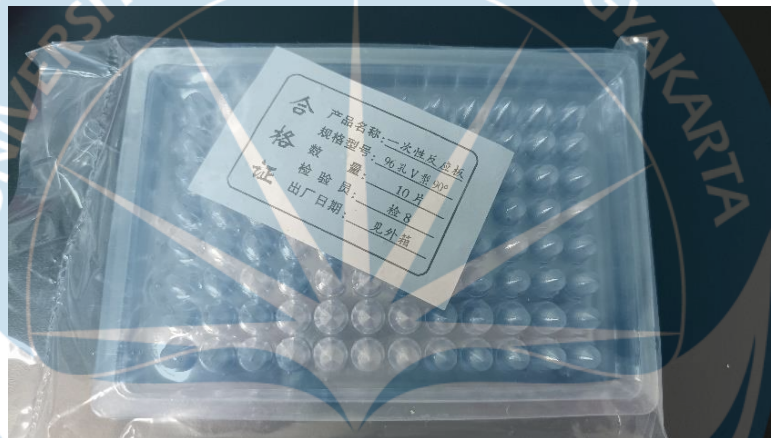
Gambar 37. Mikrotips 1000 µL



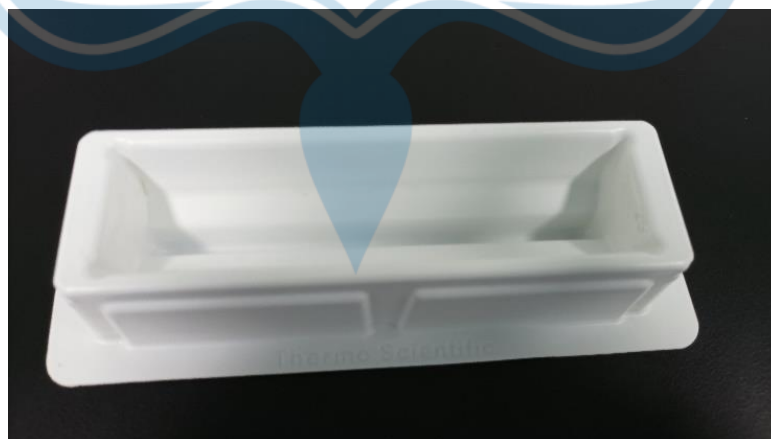
Gambar 38. Mikropipet 100, 200 dan 1000 µL



Gambar 39. Tabung konikal (Falcon) steril



Gambar 40. Flexible PVC 96 well plates U bottom



Gambar 41. Reservoir



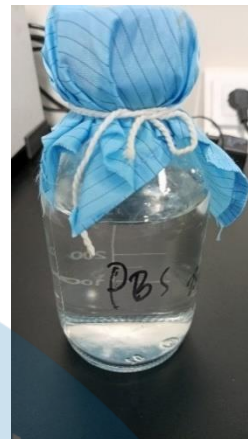
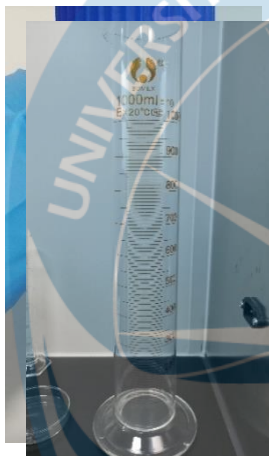
Gambar 42. *Timer*



Gambar 43. *Spidol Marker*



Gambar 44. Antigen H5N1 dan H9N2

Gambar 45. *Phosphate Buffered Saline*

Gambar 46. Gelas ukur (Bomex) 1 L



Gambar 47. Gelas beker (Iwaki) 1 L

B. Lampiran Data

Lampiran 1. Uji Normalitas

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
HAU (25°C)	.510	16	.000	.405	16	.000
HAU (37°C)	.518	16	.000	.398	16	.000

Hasil analisis statistika dengan *software* SPSS versi 21 menunjukkan data pada kelompok inkubasi 25°C dan kelompok 37°C terdistribusi tidak normal, sehingga dilanjutkan dengan uji non-parametrik yakni Uji *Friedman* dan Uji *post-hoc Kruskal-Wallis*

Lampiran 2. Hasil analisis uji *Friedman* terhadap kelompok perlakuan temperatur inkubasi 25°C dan 37°C

Test Statistics ^a		Ranks	
N	16	Mean Rank	
Chi-Square	1.000	HAU (27°C)	1.47
df	1	HAU (35°C)	1.53
Asymp. Sig.	.317		

Perlakuan temperatur inkubasi 25°C dan 37°C terhadap hasil uji hemaglutinasi tidak menunjukkan perbedaan yang signifikan berdasarkan hasil analisis statistik uji *Friedman* dengan nilai *asymp. sig.* >0,05 pada tingkat kepercayaan 95%

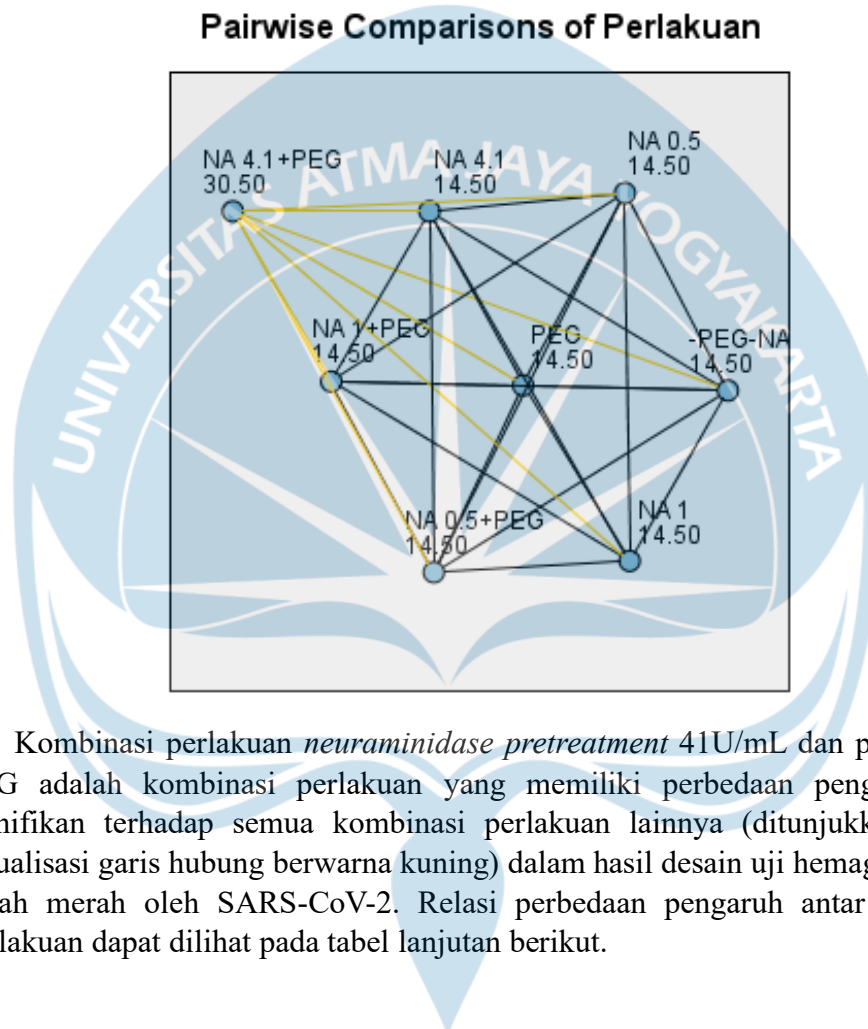
Lampiran 3. Uji *post-hoc Kruskal-Wallis*

Null Hypothesis	Test	Sig.	Decision
1 The distribution of HAU is the same across categories of Perlakuan.	Independent-Samples Kruskal-Wallis Test	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Hasil uji *post-hoc* *Kruskal-Wallis* menunjukkan ada kombinasi perlakuan yang memiliki perbedaan yang signifikan dengan kombinasi perlakuan lainnya dengan nilai *asympt. sig.* $<0,05$ pada tingkat kepercayaan 95%.

Lampiran 4. Visualisasi data *Pairwise Comparisons* dalam Uji *post-hoc* *Kruskal-Wallis*



Kombinasi perlakuan *neuraminidase pretreatment* 41U/mL dan penambahan PEG adalah kombinasi perlakuan yang memiliki perbedaan pengaruh yang signifikan terhadap semua kombinasi perlakuan lainnya (ditunjukkan dengan visualisasi garis hubung berwarna kuning) dalam hasil desain uji hemaglutinasi sel darah merah oleh SARS-CoV-2. Relasi perbedaan pengaruh antar kombinasi perlakuan dapat dilihat pada tabel lanjutan berikut.

Each node shows the sample average rank of Perlakuan.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
.PEG-NA-PEG	.000	3.808	.000	1.000	1.000
.PEG-NA-NA 0.5	.000	3.808	.000	1.000	1.000
.PEG-NA-NA 1	.000	3.808	.000	1.000	1.000
.PEG-NA-NA 4.1	.000	3.808	.000	1.000	1.000
.PEG-NA-NA 0.5+PEG	.000	3.808	.000	1.000	1.000
.PEG-NA-NA 1+PEG	.000	3.808	.000	1.000	1.000
.PEG-NA-NA 4.1+PEG	-16.000	3.808	-4.202	.000	.001
PEG-NA 0.5	.000	3.808	.000	1.000	1.000
PEG-NA 1	.000	3.808	.000	1.000	1.000
PEG-NA 4.1	.000	3.808	.000	1.000	1.000
PEG-NA 0.5+PEG	.000	3.808	.000	1.000	1.000
PEG-NA 1+PEG	.000	3.808	.000	1.000	1.000
PEG-NA 4.1+PEG	-16.000	3.808	-4.202	.000	.001
NA 0.5-NA 1	.000	3.808	.000	1.000	1.000
NA 0.5-NA 4.1	.000	3.808	.000	1.000	1.000
NA 0.5-NA 0.5+PEG	.000	3.808	.000	1.000	1.000
NA 0.5-NA 1+PEG	.000	3.808	.000	1.000	1.000
NA 0.5-NA 4.1+PEG	-16.000	3.808	-4.202	.000	.001
NA 1-NA 4.1	.000	3.808	.000	1.000	1.000
NA 1-NA 0.5+PEG	.000	3.808	.000	1.000	1.000
NA 1-NA 1+PEG	.000	3.808	.000	1.000	1.000
NA 1-NA 4.1+PEG	-16.000	3.808	-4.202	.000	.001
NA 4.1-NA 0.5+PEG	.000	3.808	.000	1.000	1.000
NA 4.1-NA 1+PEG	.000	3.808	.000	1.000	1.000
NA 4.1-NA 4.1+PEG	-16.000	3.808	-4.202	.000	.001
NA 0.5+PEG-NA 1+PEG	.000	3.808	.000	1.000	1.000
NA 0.5+PEG-NA 4.1+PEG	-16.000	3.808	-4.202	.000	.001
NA 1+PEG-NA 4.1+PEG	-16.000	3.808	-4.202	.000	.001

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Lampiran 5. Perhitungan Konsentrasi *Neuraminidase*

Neuraminidase (41 units/mg) 1,45 mg dalam kemasan ditambahkan 0,145 mL PBS
 $= (41 \text{ U/mg} \times 1,45 \text{ mg}) / 0,145 \text{ mL}$
 $= 5,945 \text{ U}/0,145 \text{ mL}$
 $= 41 \text{ U/mL}$

<i>Neuraminidase</i> (NA)		<i>Phosphate Buffered Saline</i> (PBS)	<i>Neuraminidase Working Solution</i>		
Konsentrasi awal (Unit)	Volume NA (mL)	Volume PBS (mL)	Volume Akhir (mL)	Konsentrasi Akhir (U/mL)	Konsentrasi Akhir (U/0,1 mL)
41	0,1	0	0,1	41	4,1
41	0,0243902	0,07561	0,1	10	1
41	0,0121951	0,087805	0,1	5	0,5

Konsentrasi Awal x Volume Awal = Konsentrasi Akhir x Volume Akhir

Lampiran 6. Perhitungan Konsentrasi PEG

Larutan stok PEG 15% (w/v) dibuat dengan melarutkan 2.1 g serbuk PEG (4000) dengan 14 mL PBS
 $= 15\% \times 14 \text{ mL} = 2.1 \text{ g}$

PEG Stok		PEG Stok Pengenceran (% w/v)	
Konsentrasi awal %	Volume awal PEG (μL)	Volume Akhir (μL)	Konsentrasi Akhir %
15	66,67	1000	1
15	133,33	1000	2
15	200	1000	3
15	266,66	1000	4
15	333,33	1000	5
15	400	1000	6
15	326,66	700	7
15	373,33	700	8
15	420	700	9
15	466,66	700	10
15	513,33	700	11
15	560	700	12
15	606,66	700	13
15	653,33	700	14

Konsentrasi Awal x Volume Awal = Konsentrasi Akhir x Volume Akhir