Immune Adherence Hemagglutination

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Procedure

1. Place 30µL of the immune complex[note 1] in HBS++[note 2] on a U-bottom plate
2. Add 30µL of normal human plasma (NHP)[note 3]
3. Incubate the plate at 37˚C for 30min
4. Add 30µL 35mM N-Acetylcysteine (Nac) in PBS[note 4]
5. Add 30µL 1.5% human erythrocyte in PBS[note 5]
6. Incubate the plate at room temperature (RT) for 60min
7. Interpret the results visually (scoring from 0 – 4+)

Note 1

- A variety of immune complexes may be used including:
  - Heat-aggregated plasma (HAP)
    - NHP heated at 63˚C on a heat block for 30min
  - Heat-aggregated immunoglobulin (HAI)
    - Prepared 10mg/mL, then heated at 63˚C on a heat block for 30min
  - TT-HTIG mixture
    - Equal volumes of 1:10 tetanus toxoid (TT) and 1:8000 human tetanus immunoglobulin (HTIG) are mixed, then incubated at 37˚C for 30 min
  - COVID peptide + COVID sera mixture
    - Equal volumes of 400mcg/mL COVID peptide and COVID sera are mixed, then incubated at 37˚C for 30 min
  - Inada et al (1981) used the following immune complexes as standards
    - Heat aggregated human IgG (HAG) – prepared 10mg/mL, 63C, 30min (when testing, start @ 500mcg/mL)
    - 25uL 1:800 rabbit antihuman IgG serum + 25uL serially diluted human IgG (starting 64mcg/mL), 37C, 60min

Note 2

- HBS++ is HEPES buffered saline (pH 7.4) supplemented with magnesium and calcium. Gelatin was removed to promote buffer longevity
- This method works with gelatin-HEPES buffer supplemented with magnesium and calcium (GHB++) as well
- In the original procedure by Inada et al. (1981), gelatin-veronal buffer supplemented with magnesium and calcium (GVB++) was used

Note 3

- This step adds the complement source
- This method works with normal human sera (NHS) as well
- In the original procedure by Inada et al. (1981), guinea pig sera was used

Note 4

- The purpose of this step is to stabilize C3b on the erythrocyte surface
- In my experiments, I dilute 171µL Nac to 3mL with PBS
- Notice the shift from HBS++ to PBS. Complement activation ends at this step because of dilution of Mg^{2+} & Ca^{2+}.
- PBS is cheaper than HBS. Theoretically at least, HBS may be used in place of PBS. No problems with the use of PBS have been encountered. Potential problems include the precipitation of calcium phosphate, although this occurs at more basic pH (with an optimum of 10–12)
- I use 100mg/mL N-acetylcysteine solution for injection (IM/IV) ampules
- This method works with N-acetylcysteine 600mg effervescent tablets as well, but note that it contains various excipients that may interfere, such as starches that could potentially activate complement
- In the original procedure by Inada et al. (1981), 20mM dithiothreitol (DTT) was used. I replaced it with Nac using a 0.98:1.6 ratio roughly based on this paper: https://www.ncbi.nlm.nih.gov › articles › PMC1475835

Note 5

- For my experiments, I dilute 165µL of human E in 11mL PBS (for use with 2 plates) or 60 µL human E in 4mL.
- I use human E from whole blood containing CPDA-1 as anticoagulant

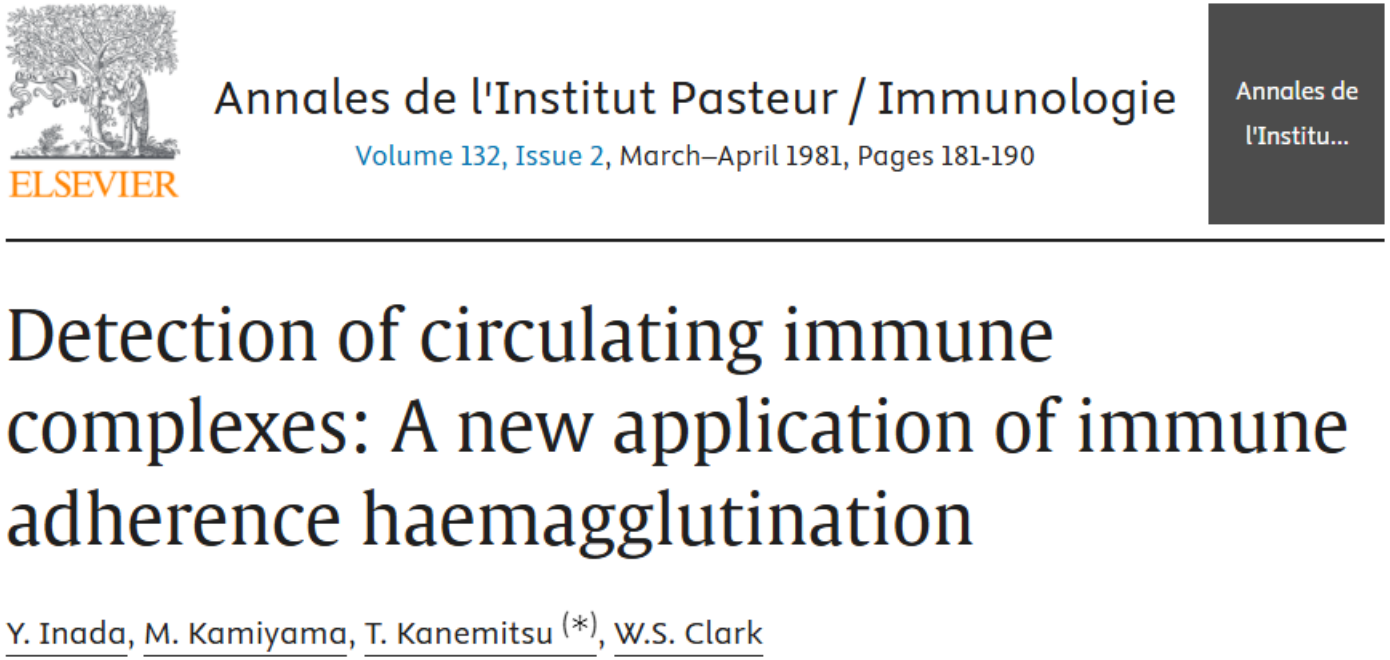
Note 4 & 5

- To abrogate the effects of complement, EDTA-PBS* may be used in lieu of PBS for steps 4 & 5. It works via chelation of divalent cations.
- In the original procedure by Inada et al. (1981), 10mM EDTA-GVB was used.

- *I have used a variety of EDTA concentrations, ranging from 4mM to 40mM

Reference

- https://sci-hub.ru/https://doi.org/10.1016/0769-2625(81)90026-X



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Detection of circulating immune complexes: A new application of immune adherence haemagglutination

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Sample results

A

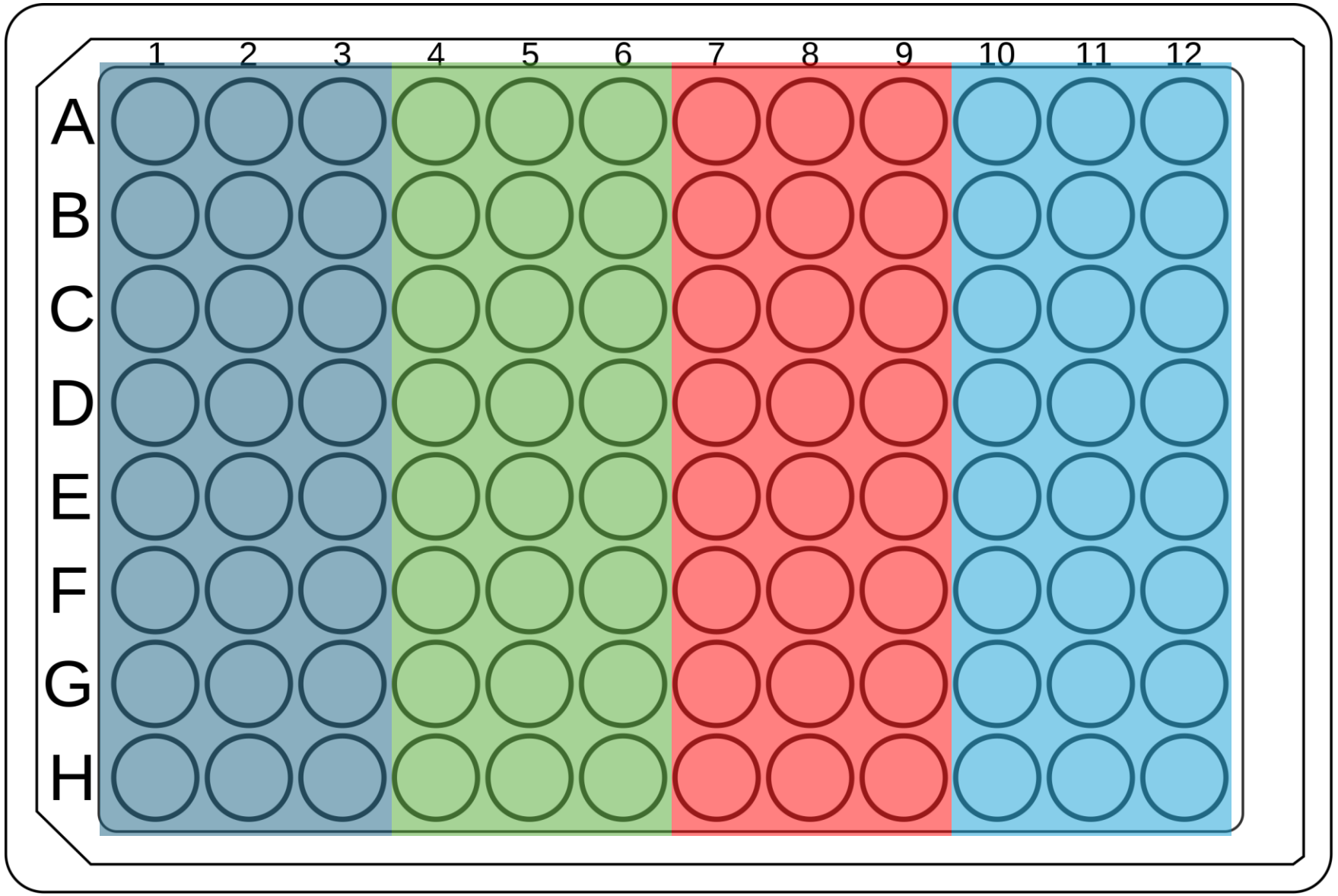
HAI (4mcg/mL)

7.5% papain 35mMNac PBS

HAP (100%)

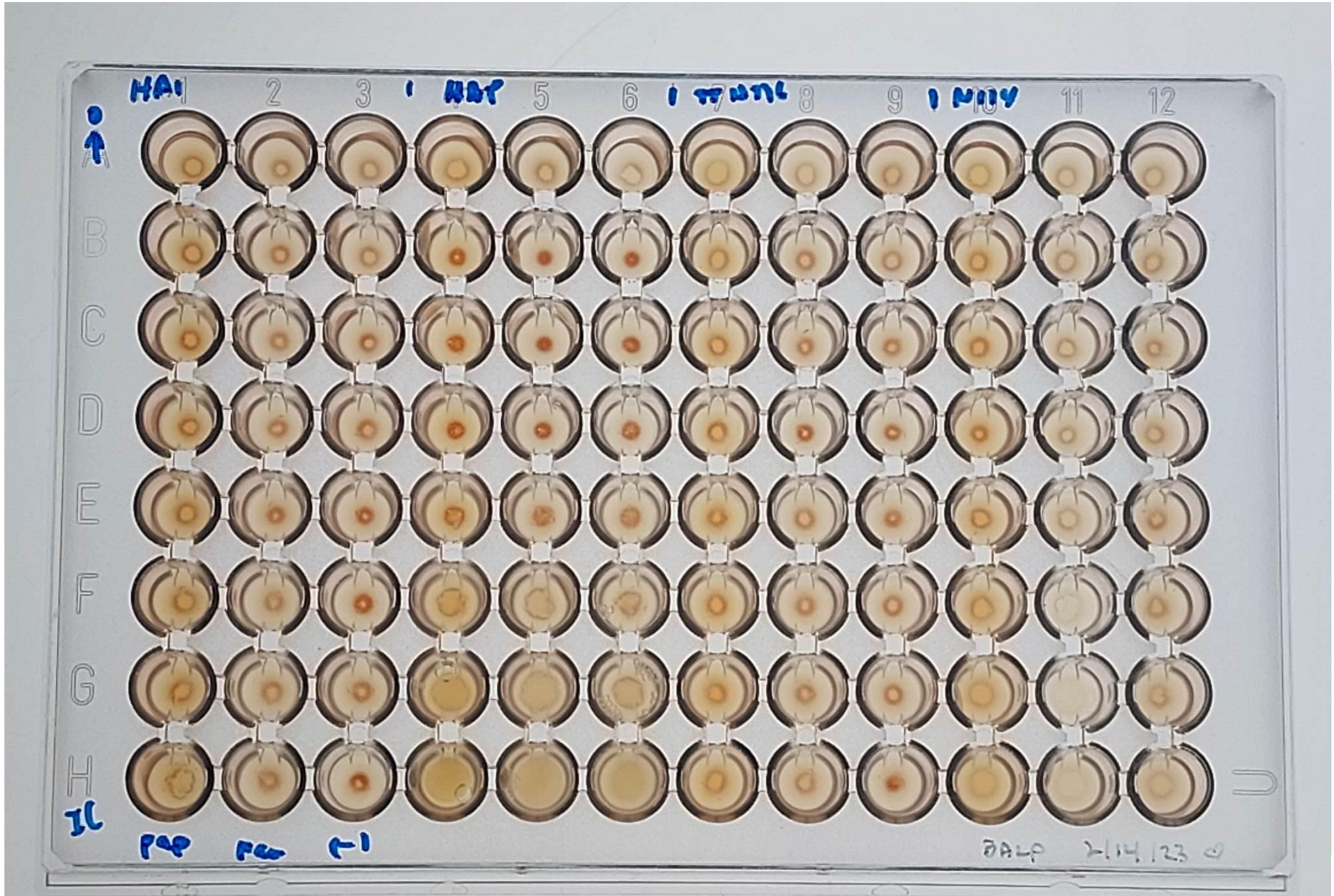
TT+HTIG (1:10 + 1:8000)

N114 (400mcg/mL)



A

HAI	HAP	TT+HTIG	N114
(4mcg/mL)	(100%)	(1:10 + 1:8000)	(400mcg/mL)
7.5% papain 35mMNac PBS			



60 min