Detection of Type 1 Diabetes Using Antibody Detection by Agglutination-PCR on the ADAP STAR Assay Ready Workstation

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Introduction

Antibody detection assays, such as ELISA, immunoprecipitation, and radioimmunoassays are important tools to diagnose diseases such as Type 1 Diabetes (T1D)[1–3]. When performed manually, these common yet labor-intensive assays can take up to 22 hours and are subject to inconsistency between technicians and laboratories. In addition, gold standard radioimmunoassays require radioactive reagents, which increase assay costs and risk of personnel exposure to hazardous reagents, and offer minimal multiplexing capability. As a result, they are not feasible for large population antibody screening.

Antibody Detection by Agglutination-PCR (ADAP) differs from traditional immunoassays in that it is a pure solution-phase assay and well-suited to detecting sensitive antibodies. The assay utilizes a pair of antigen-DNA conjugates to probe the sample target antibodies. If present, agglutination between the antibody and antigen, and ligation, leads to formation of a specific DNA amplicon detected by qPCR.

In this application note, we describe multiplexed ADAP detection of Insulin (Ins), Islet Antigen 2 (IA-2), and Glutamic Acid Decarboxylase (GAD) using the ADAP T1D reagent chemistry kit and readily available quantitative (qPCR) instruments. The ADAP T1D reagent chemistry kit consists of four reagents, each labeled for agglutination, ligation, pre-amplification, and qPCR primer quantification, along with positive and negative controls. Up to four antibodies may be detected per sample in a 384-well quantification plate. Using the ADAP T1D reagent chemistry kit, the autoantibodies can be detected in the blood and serum of pre-symptomatic individuals with T1D, thus offering an avenue for early disease management, such as timely glycemic control, to prevent metabolic complications and reduce the onset of potentially fatal diabetes ketoacidosis.

The workflow is automated using the ADAP STAR assay ready workstation from Hamilton Company (see workflow and deck layout at end). The ADAP STAR is based on the Company's Microlab® STAR™ liquid handling workstation, and is configured with CO-RE® gripper paddles and the iSWAP robotic transport arm for hands-free plate and lid movements. Microlab VENUS® software in ADAP STAR was optimized and pre-programmed for all steps. Using this automated workflow, up to 80 samples, along with necessary controls, may be analyzed in one automated run. We demonstrate that the ADAP STAR enables increased throughput and facilitates sample-to-answer within three hours.

Benefits-Based Highlights

- Enable sample-sparing analysis of precious samples.
- Achieve sensitive and specific antibody detection results.
- Eliminate time-consuming and repetitive manual pipetting.
- Reduce or eliminate risks of human error and variability to ensure consistency and quality of downstream results.

Materials and Methods

The ADAP STAR was manually loaded with the following consumables:

- 96- and 384-well PCR frame plates (Hamilton, P/N 814300, 814302)
- 50 µL Conductive Non-Sterile Filter Tips (Hamilton, P/N 235948)
- 300 µL Conductive Non-Sterile Tips (Hamilton, P/N 235903)
- PCR Comfort Lid (Hamilton, P/N 814300)
- Optically Clear Plate Seals (Hamilton, P/N 67765-01)

Reagents from the ADAP T1D reagent chemistry kit (Enable Biosciences, Cat# EBTD0001) were manually prepared according to manufacturer instructions. Agglutination mix, ligation mix, pre-amplification mix, and the three qPCR-primer mixes from the ADAP T1D reagent chemistry kit were added to 1.5 mL conical microcentrifuge tubes on the CPAC, and ligation mix and dilution water were added to a chilled 12-column reagent reservoir (Agilent, Cat# 201256-100).

The ADAP Assay was performed manually and on the Hamilton Microlab STAR as follows:

1. 8 µL of agglutination mix and 4 µL of serum sample were added to a 384-well frame plate and transferred to the 384-well ODTC and incubated at 37ºC for 30 min.
2. 4 µL of the resulting mix was added to a 96-well frame plate along with 116 µL of ligation mix and transferred to the 96-well ODTC and incubated at 30ºC for 15 min.
3. 25 µL of the resulting mix was added to a 96-well frame plate along with 25 µL of pre-amplification mix and transferred to the 96-well ODTC and subjected to thermocycling (13 cycles of PCR, cycling between 95ºC and 56ºC for a total of approximately 40 min.).
4. The amplified product was diluted 20-fold using molecular biology grade water (Corning, Cat# 46-000-CM) and 8.5 µL of the diluted product was added to 11.5 µL of each individual qPCR-primer mix.
5. The final solution was sealed using the plate sealer for subsequent qPCR quantification on a CFX384 Touch™ Real-Time Detection System (Bio-Rad Laboratories, Hercules, CA).

Results and Discussion

Intra-Assay Precision

As can be seen in Table 1, the intra-assay precision was calculated for both the manual and automated ADAP assays. Mean, standard deviation, and coefficient of variation (%CV) of serum samples positive for T1D antibodies were calculated for an N=5 for the manual and N=20 for the automated ADAP assay. ΔC_T is defined as the difference in cycle threshold (C_T) between blank sample and actual sample and used as a performance characteristic. Due to the nature of this assay, the %CV of ΔC_T for negative samples is not representative of assay variation and not considered as an indicator of performance characteristics. The intra-assay variations were in-line with the industry standard for method validation developed by the US Department of Health and Human Services, Food and Drug Administration[4].

Table 1: Comparison of Intra-Assay Precision in Samples Processed Using Manual and Automated Methods

	Manual ADAP Workflow						Automated ADAP Workflow					
	Ins		IA-2		GAD		Ins		IA-2		GAD	
Mean	1.90	0.40	8.10	-0.30	6.40	0.80	1.50	-0.30	7.20	-0.50	5.00	-0.60
SD	0.20	0.05	0.10	0.20	0.10	0.20	0.30	0.30	0.30	0.30	0.40	0.50
%CV	9.00	–	1.00	–	1.00	–	21.00	–	5.00	–	8.00	–

1. *Atkinson, M.A.; Eisenbarth, G.S.; Michels, A.W. Type 1 diabetes.* Lancet. *2014, 383(9911), 69–82.*
2. *Chiang, J.L.; Kirkman, M.S.; Laffel, L.M.; et al. Type 1 diabetes through the life span: A position statement of the American Diabetes Association.* Diabetes Care. *2014, 37(7), 2034–2054.*
3. *Miao, D.; Steck, A.K.; Zhang, L.; et al. Electrochemiluminescence assays for insulin and glutamic acid decarboxylase autoantibodies improve prediction of type 1 diabetes risk.* Diabetes Technol Ther. *2015, 17(2), 119–127.*
4. *U.S. Department of Health and Human Services, Food and Drug Administration. Bioanalytical Method Validation: Guidance for Industry. 2018, May.*

Inter-Assay Precision

The inter-assay precision was calculated for both the manual and automated ADAP assays (Table 2). Mean, standard deviation, and coefficient of variation (%CV) of serum samples positive for T1D antibodies were calculated for an N=3 for the manual and N=3 for the automated ADAP assay. ΔC_T is defined as the difference in cycle threshold (C_T) between blank sample and actual sample and used as the performance characteristic. Due to the nature of this assay, the %CV of ΔC_T for negative samples is not representative of assay variation and not considered as an indicator of performance characteristics. The inter-assay variations were in-line with the industry standard for method validation developed by the US Department of Health and Human Services, Food and Drug Administration[4].

Clinical Sensitivity/Specificity

Sensitivity was calculated as the number of T1D samples positive for at least one autoantibody in the ADAP assay divided by the total number of positive T1D samples multiplied by 100. Specificity was calculated as the number of non-T1D samples negative for all three autoantibodies in the ADAP assay divided by the total number of non-T1D samples multiplied by 100. These calculations were performed on both the manual and automated methods and can be found in Table 3. The results affirmed high sensitivity and specificity of the methods.

Correlation

To ensure concordant results, 60 clinical specimens were assayed manually and on the ADAP STAR assay ready workstation. As determined using the data plotted in Figure 1, the correlation coefficients for three autoantibodies were GAD = 0.98, Ins = 0.94, and IA-2 = 0.96. These results affirmed the two methods were highly correlated.

Table 2: Comparison of Inter-Assay Precision in Samples Processed Using Manual and Automated Methods

	Manual ADAP Workflow						Automated ADAP Workflow					
	Ins		IA-2		GAD		Ins		IA-2		GAD	
Mean	1.80	0.40	7.80	-0.20	6.20	0.80	1.60	-0.20	7.30	-0.20	5.10	-0.50
SD	0.10	0.08	0.30	0.20	0.20	0.09	0.20	0.30	0.20	0.30	0.10	0.20
%CV	5.00	–	4.00	–	4.00	–	10.00	–	2.00	–	2.00	–

Table 3: Comparison of Clinical Sensitivity and Specificity in Samples Processed Using Manual and Automated Methods

	Manual ADAP Workflow	Automated ADAP Workflow
Clinical Sensitivity	95%	95%
Clinical Specificity	98%	98%

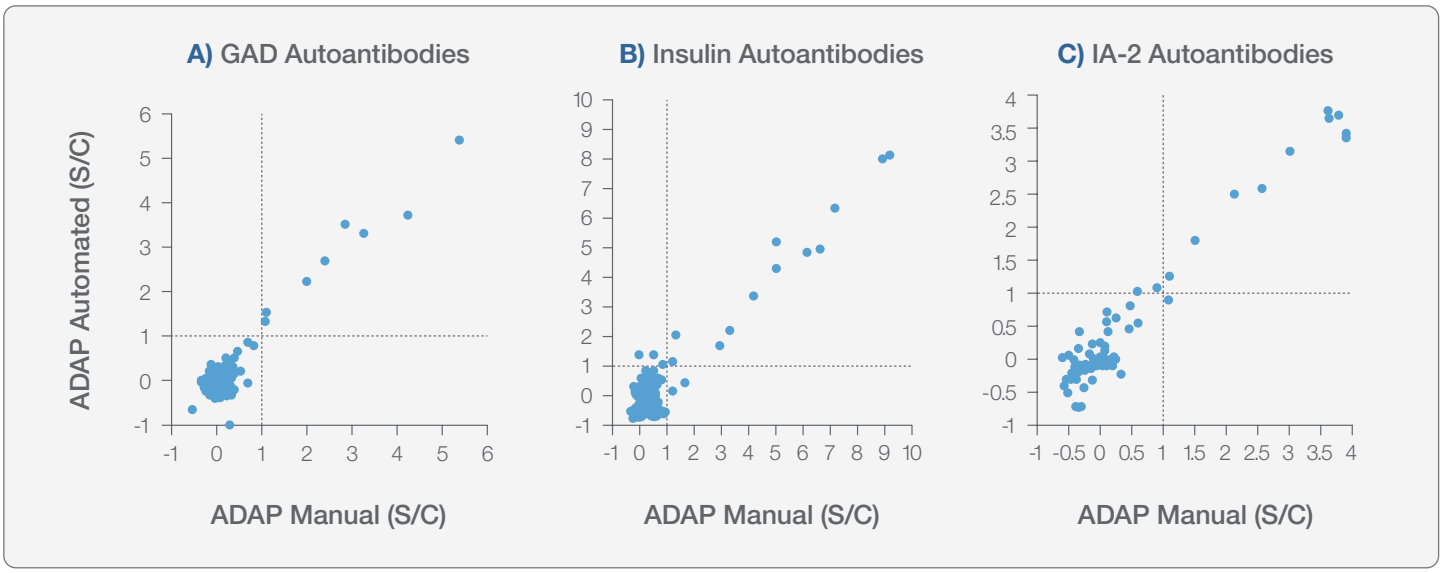
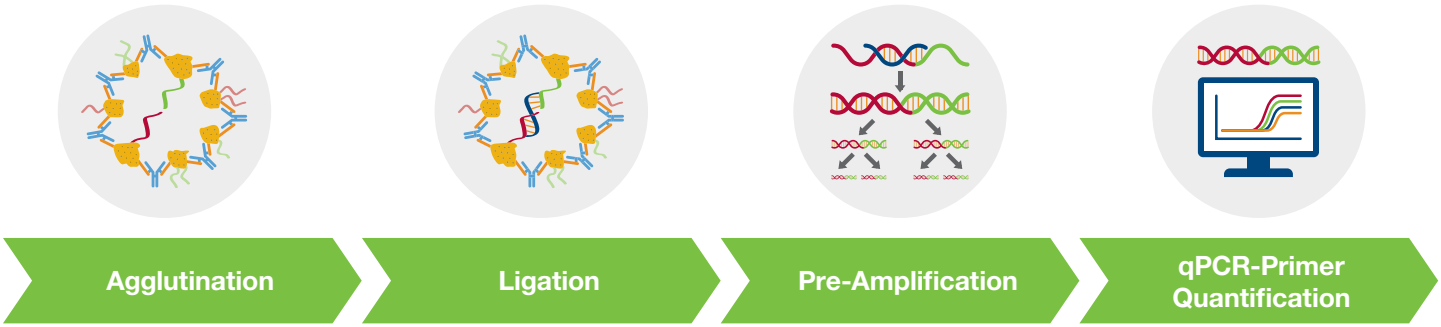


Figure 1. Correlation of (A) GAD; (B) Ins; and (C) IA-autoantibody values between manual and automated methods. N=60.

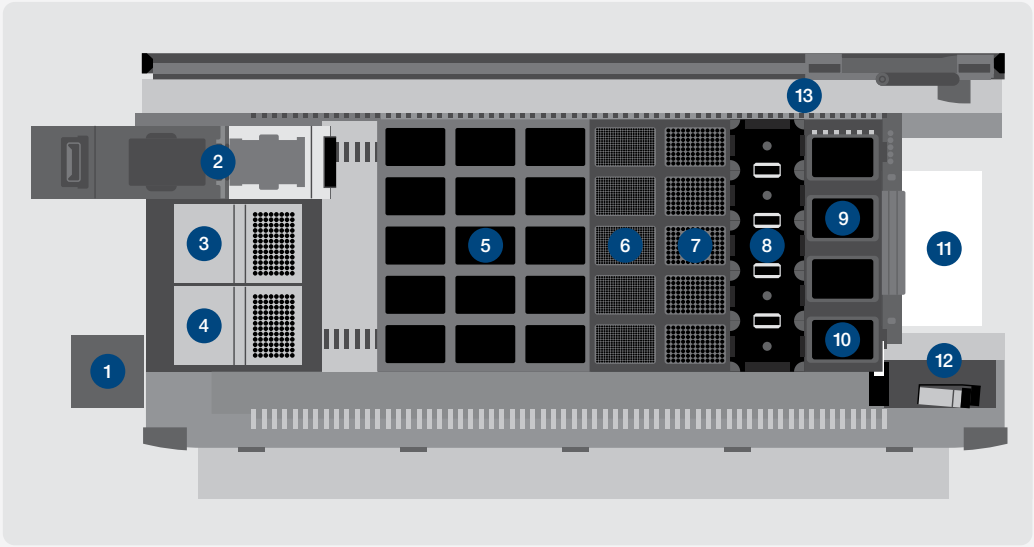
Conclusion

The ADAP T1D reagent chemistry kit can provide multiplexed, highly specific, and selective islet autoantibody results within two hours while using readily available quantitative (qPCR) instruments. Enable Biosciences and Hamilton collaboratively developed the fully automated ADAP STAR assay ready workstation to robustly perform all steps in the ADAP antibody detection assay in a hands-free, high-throughput manner. After loading samples on the ADAP STAR, there is no need for manual intervention until the final quantification plate is sealed. Results obtained using the automated workflow were comparable to or better than results obtained through manual methods. Additionally, the ADAP STAR vastly reduces the exposure to potentially pathogenic bodily fluids and supports consistent results by avoiding human error in pipetting.

Workflow



ADAP STAR Deck Layout for the Type 1 Diabetes Assay



1. 96 MPH Waste
2. Plate Sealer
3. 96-well Thermal Cycler
4. 384-well Thermal Cycler
5. 50 µL and 300 µL Pipette Tips
6. 384-well PCR Plate Positions
7. 96-well PCR Plate Positions
8. Sample Plate Carrier
9. Cold Plate Air Cooled (CPAC) Devices
10. Comfort Lid Stack
11. Waste
12. Autoload
13. iSWAP

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