Protocol Number:	Q170886
Protocol Title:	Cognoa ASD Diagnosis Aid Validation Study
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cognoa	**Plan** **Title:** Statistical Analysis Plan Cognoa ASD Diagnosis Aid Validation Study	Document No.: PLN-004
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Doc Title:	Statistical Analysis Plan version 1.4.7 Cognoa ASD Diagnosis Aid Validation Study		
Study Protocol Number:	Q170886		
Approvals			
Role on Project Team	**Name**	**Signature**	**Date**
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Cognoa, the Study Sponsor, wants to make modifications to the clinical study protocol during the course of the clinical investigation.

The modification to the clinical protocol consists in increasing the study sample size, which will increase the statistical power of the investigation and confidence in reaching the defined primary endpoints.

The modification to the protocol does not affect
(I) the validity of data or information resulting from the completion of the approved protocol, or the relationship of likely patient risk to benefit relied upon to approve the protocol;
(II) the scientific soundness of the investigational plan; or
(III) the rights, safety, or welfare of the human subjects involved in the investigation.

Enrollment in the study has increased steadily across the sites, which has triggered Cognoa's decision to increase the sample size. The cost-benefit ratio of increasing the sample size of this study balances out the risk of having to conduct another study to obtain more evidence.

This is a very low risk study, exempt from the IDE requirements. It is also a blinded study and the Sponsor has no insight on the study results. The data is being handled by Statking Clinical Services according to the Data Management Plan v1.0 dated November 5, 2019. 600 subjects have been enrolled, 217 completed their specialist visit and no adverse event has been recorded as of this date.

REVISION HISTORY

Change Request Number	Revision	Effective Date	Change
NA	C	Feb 3, 2020	Increase sample size

STATISTICAL ANALYSIS PLAN

Cognoa ASD Diagnosis Aid Validation Study

Cognoa, Inc.

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Prepared by:
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SAP version 1.4.7

1 INTRODUCTION

1.1 Background

This statistical analysis plan is in reference to the clinical study outlined in *Cognoa ASD Diagnosis Aid Validation Study* ("the Protocol").

1.2 Study objective

To assess the ability of the Cognoa ASD Diagnosis Aid ("the Device") to aid in the diagnosis of ASD by comparing its diagnostic output with the clinical reference standard (specified in section *V. Study Procedures* of the Protocol), in terms of positive and negative predictive values, coverage, sensitivity, and specificity.

2 STUDY METHODS

2.1 Study Design

The study is a blinded, multi-site, prospective, active comparator cohort study. For more details on the design, refer to the Protocol.

All study participants and assessors will be blinded to the results of the device. Analysts at Cognoa, Inc. will be blinded to the diagnosis results for study participants and no analysis will be performed using the diagnosis results until the conclusion of the study.

Blind will remain unbroken until the completion of the study, including all data collection, at which point statistical analysis of the endpoints will be performed. For the full specification of the analysis of the endpoints, refer to section *5.1 Analysis Methods* of this document.

The device has tunable parameters, including underlying machine learning models, operational decision thresholds, and the agents involved. These elements are specified prior to the commencement of the study and cannot be changed during the study.

All aspects of the reference standard are specified prior to the commencement of the study and cannot be changed during the study. For specifications of the reference standard, refer to section *V. Study Procedures* of the Protocol.

2.2 Primary Endpoints

1. Composite endpoint consisting of:
    a. Positive Predictive Value (PPV) of the device, greater than 65%
    b. Negative Predictive Value (NPV) of the device, greater than 85%

2. Proportion of all subjects receiving "no result" output

The first, composite primary endpoint will be evaluated for success or failure as a group. For sample size and power analysis purposes, the overall error rate is controlled on a per-endpoint basis. For full specifications of the analysis of the primary endpoints, refer to section *5.1 Analysis Methods* of this document. For full sample size calculations, refer to section *5.5 Sample Size and Power Analysis* of this document.

2.3 Secondary Endpoints

1. Sensitivity to ASD of the device
2. Specificity to ASD of the device

These secondary endpoints will be analyzed by reporting the estimate of the specified metric as well as its 95% confidence interval. The trial will not be powered for rigorous statistical tests of the secondary endpoints. For full specifications of the analysis of the secondary endpoints, refer to section *5.1 Analysis Methods* of this document.

2.4 Sample size

The sample size for the study will be 725. For full sample size calculations, refer to section *5.5 Sample Size and Power Analysis* of this document.

2.5 Statistical Framework

The first primary endpoint evaluates whether the device meets the pre-specified performance goals. This will be determined using two-sided 95% statistical confidence intervals. Other endpoints include the reporting of a number of metrics about the device's performance using 95% confidence intervals. For full specifications of the analysis of the endpoints, refer to section *5.1 Analysis Methods* of this document.

2.6 Mitigation of Bias

2.6.1 Verification bias:
No verification bias is expected to be present. All study participants will receive the reference standard diagnosis and be assessed by the device.

2.6.2 Selection bias:
No selection bias is expected to be present. The study enrollment criteria are set to match the intended indications for use of the device. Specifically, the parental level of concern for possible autism confirmed by the HCP and allowed age range are managed. For more details, refer to section *IV. Study Subjects* of the Protocol.

2.6.3 Spectrum bias:

No spectrum bias is expected to be present. The study design adopts open enrollment that is expected to enroll children from these relevant subgroups at the rates at which they are present in the target population:

- Children who are developing normally
- Children with ASD
- Children with other neurodevelopmental conditions such as ADHD, speech and language delay, etc.
- Children with both ASD and one or more other neurodevelopmental conditions

The proportions of children in the sample with these conditions are expected to match the proportions of children in the target population with these conditions, and the observed proportions will be reported.

2.6.4 Training bias:
No training bias is expected to be present. Every caregiver will undergo the device assessment only once, and all previous Cognoa users are excluded from the study. Therefore, no individual user will have the opportunity to change their ability with the device over the course of the study. Health Care Provider (HCP) users will be trained on using the device prior to the commencement of the study, in order to reduce the risk that a HCP changes how they use the device over the course of the study.

3 STUDY POPULATION

The study population is intended to be representative of the population of children, aged between 18 months and 72 months, who visit their primary HCP for standard check-ups such as wellness checks and about whom the HCP or the caregiver has concern for developmental delay. For more details, refer to section 7. *Study Population* of the Protocol.

In the study population, we expect the prevalence of ASD to be higher than the prevalence of ASD in the general population. Based on prior investigations performed by Cognoa, Inc., we expect the prevalence of ASD in the study population to be approximately 24%.

We expect the study population to match the population of the United States in terms of race and ethnicity proportions.

4 STATISTICAL PRINCIPLES

4.1 Confidence Intervals and P-values

In the analysis of the primary endpoints, 3 two-sided confidence intervals will be computed. Each endpoint will be evaluated at a 95% confidence level. The analysis of the first primary endpoint includes the computation of two distinct confidence intervals and two minimally

acceptable values that must be surpassed for the endpoint's performance goal to be considered met. In order to achieve a joint 95% confidence level for the entire endpoint, each of the two confidence intervals will be computed with a 95% confidence level and the lower end of the interval checked against the minimum acceptable value. This ensures a joint 95% confidence level on the conclusion that both measures evaluated in the first primary endpoint meet their minimum acceptable values. The second primary endpoint consists of a single confidence interval, which will also be 95% and two-sided.

All other confidence intervals presented will also be 95% and two-sided.

For full specifications of the analysis of the endpoints, refer to section *5.1 Analysis Methods* of this document.

4.2 Analysis Populations

The analysis will be performed on an intention-to-diagnose basis [2]. The analysis population will include all participants. For details on how dropouts, missing data, and protocol noncompliance will be analyzed, refer to section *5.2 Handling of Dropouts and Missing Data* of this document.

5 STATISTICAL ANALYSIS METHODOLOGY

The response of the device is categorical. It reports that a subject is Positive for ASD ("+ASD") or Negative for ASD ("-ASD"). Additionally, the device has the option to "abstain" from classification when the sample is not evaluable ("no result").

The following contingency table describes all the possible combinations of the device diagnostic output and clinician panel determination (the reference standard). The nomenclature outlined here is used in the following sections to specify the metrics used in the evaluation of the endpoints.

		Clinician Panel Determination (Reference Standard)	
		Positive ASD	Negative ASD
Device Diagnostic Output	+ASD	True Positive (TP)	False Positive (FP)
	-ASD	False Negative (FN)	True Negative (TN)
	No Result	No Result Positive (NRP)	No Result Negative (NRN)

5.1 Analysis Methods

5.1.1 Analysis of the Primary Endpoints

The Predictive Values of the device outcomes will be computed as:

Device Outcome	Probability of Interest	Computation	Minimally Acceptable Value
+ASD	PPV = P(Ref=+ASD | Device=+ASD)	$\frac{TP}{TP + FP}$	0.65
-ASD	NPV = P(Ref=-ASD | Device=-ASD)	$\frac{TN}{TN + FN}$	0.85

Two-sided 95% confidence intervals of each metric will be computed and reported. If both confidence intervals lie entirely above the minimally acceptable values, the endpoint will be considered to be met.

The proportion of all subjects who receive a "no result" outcome will be computed as:

Device Outcome	Probability of Interest	Computation
no result	P(Device=no result)	$\frac{NRP + NRN}{\Sigma all}$

A two-sided 95% confidence interval around the metric will be computed and reported.

Note that the values used in the computation of the metrics used in the analysis of the primary endpoints are only defined for data-complete subjects. Therefore, in the analysis of the primary endpoints only subjects who complete the device assessment will be included. Additional analyses will be performed on subjects who drop out, as specified in section *5.2 Handling of Dropouts and Missing Data* of this document.

5.1.2 Analysis of the Secondary Endpoints

The Sensitivity and Specificity of the device will be computed as:

Metric	Probability of Interest	Computation
ASD Sensitivity	P(Cognoa=+ASD | Ref=+ASD)	$\frac{TP}{TP + FN}$
ASD Specificity	P(Cognoa=-ASD | Ref=-ASD)	$\frac{TN}{TN + FP}$

Two-sided 95% confidence intervals of each metric will be computed and reported.

5.1.3 Additional Analyses

Additionally, in order to quantify the makeup of the group of subjects for whom the Device provides no result, the following proportions will be computed:

Metric	Probability of Interest	Computation
Proportion of "no result" with +ASD	P(Ref=+ASD | Cognoa=no result)	$\frac{NRP}{NRP + NRN}$
Proportion of "no result" with -ASD	P(Ref=-ASD | Cognoa=no result)	$\frac{NRN}{NRP + NRN}$

Two-sided 95% confidence intervals of each metric will be computed and reported.

5.2 Handling of Dropouts and Missing Data

The statistical analysis will be performed on an intention-to-diagnose basis [2]. Therefore subjects, once they have completed the consent process, will not be dropped from the study analysis. If a subject fails to complete the study for any reason, they will be retained in record-keeping and reported in the final analysis.

For any subjects that are missing the reference standard, both worst-case imputation and best-case imputation will be performed. Specifically, 2 sets of performance metrics and their 95% confidence intervals will be reported. They are as follows:

1. One set of outcome metrics will be reported with the missing reference standards assumed to be opposite to the device response ("worst-case").
2. One set of outcome metrics will be reported with the missing reference standards assumed to be the same as the device response ("best-case").

5.3 Covariate & Subgroup Analysis

Analysis of several covariates will be performed [3]. Specifically, the descriptive statistics for covariates of age, sex, race, ethnicity, parental level of education, and the clinical site of

enrollment will all be tabulated separately. The performance of the device will be reported separately for each covariate group and separate two-sided 95% confidence intervals will be computed and reported. However, the study will not be powered for any statistical inference on the covariates.

5.4 Sample size and power analysis

Cognoa plans to enroll 725 subjects in total. In prior studies of the device, dropout was identified as a potentially significant risk. Consequently, with a dropout rate of up to 25%, this total enrollment will ensure a minimum of 525 study completers.

In the intended population of use for the Cognoa device, based on prior studies with the device, we expect approximately 6% of subjects to be labeled +ASD by the device, 63% to be labeled -ASD, and 31% to get no result. With 525 study completers, we would expect approximately 32 to be labeled +ASD by the device, approximately 331 to be labeled -ASD, and approximately 163 to get no result. The following table summarizes these expected enrollment numbers.

Category	Expected Number of Subjects
Total Enrollment	725
Study Completers	544
Cognoa Test +ASD	33
Cognoa Test -ASD	343
Cognoa Test No Result	169

With these expected numbers of subjects, if 27 or more of the anticipated 33 Cognoa test positive subjects are found to truly be positive according to the reference standard then the PPV 95% confidence interval will have lower bound 68.7% and the primary endpoint's PPV performance goal will be met. Similarly, if 304 or more of the anticipated 343 Cognoa test negative subjects are found to truly be negative according to the reference standard then the NPV 95% confidence interval will have lower bound 85.3% and the primary endpoint's NPV performance goal will be met.

Cognoa expects the device to demonstrate PPV greater than 90% and NPV greater than 92%. With 90% PPV, there is a 96% chance that 27 or more of the anticipated 33 Cognoa test positive subjects will be found to truly be positive according to the reference standard. With 92% NPV, there is a 99% chance that 304 or more of the anticipated 343 Cognoa test negative subjects will be found to truly be negative according to the reference standard.

Therefore, the enrollment plan outlined above has greater than 80% power at a 95% confidence level.

Additionally, with the expected minimum 544 study completers we would expect approximately 169 to get no result from the Cognoa device and a "no result" proportion 95% confidence interval with range no greater than $\pm 7.5\%$.

5.6 Statistical software

Statistical analysis will be performed using Python and R.

7 REFERENCES

1 Design Considerations for Pivotal Clinical Investigations for Medical Devices.

2 Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests.

3 Evaluation and Reporting of Age-, Race-, and Ethnicity-Specific Data in Medical Device Clinical Studies

Cognoa Dx SAP - 1.4.7

Final Audit Report 2020-02-04

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